

Setback for creation science

Last week's decision by the US Supreme Court on the teaching of evolution is a splendid affirmation of the cause of reason, but that does not mean that nothing more will be heard of the creationists.

THE US Supreme Court may merely have followed common sense last week in striking down as unconstitutional Louisiana's law requiring that "creation science" should have equal time with evolution in the school curriculum, but that should not diminish the importance of the occasion. What if the decision had gone the other way? It is likely that several southern states would have followed Louisiana into self-ordained darkness.

Worse still, states well away from the Bible Belt would have had to suffer the debilitating clamour of the small but vociferous army of the zealous forever confronting data with the blend of fancy and whimsy that, to creationists, is evidence. That danger will now recede, especially because of the sharpness of the Supreme Court's majority decision, which called Louisiana's pretence that its demand for equal time was based on its concern for academic freedom a "sham". But it would be wrong to think that the last has now been heard from the creationists.

Other legal issues remain to be decided. Later this month, an appeal court will decide whether to intervene in a case in which an Alabama judge has ruled that certain textbooks should be banned because their darwinian content is tantamount to teaching the "religion" of "secular humanism". Later, there will be a similar inquiry into the decision of a judge in Tennessee that fundamentalist parents might withdraw their children from classes at which materials considered to be "anti-Christian" would be read, which is a more subtle question. Yet the case now decided by the Supreme Court, by the generality of the claims embodied in Louisiana's "Balanced Treatment Act", is far more important. Even so, it will be noticed, two out of nine justices dissented from the majority opinion.

That is one reason why, even now that the Supreme Court has done the decent thing, the problem of the creationists should be given more careful and even more measured attention that it may appear to deserve. In the nineteenth century, the issue was simple if incapable of resolution: darwinism was incoherently opposed by people saying they preferred to believe in *Genesis*. In its modern form, the dispute more directly concerns the validity of scientific inference; it drags on precisely because science itself is now the first to emphasize the uncertainties of the process of inference. In Sir Karl Popper's idiom, people no longer reach conclusions, but test hypotheses, which gives 'creation science' its opportunity. For, as the milder creationists are quick to say, if the theory of evolution is merely a hypothesis, why should other radically different hypotheses, the Bible stories for example, be ruled out?

Questions such as these need more careful answers than they are usually given. Contrary to what the creationists say, the theory of evolution is not a dogma, but an incomplete model of the mechanism by which known life-forms have evolved. Its value stems from its success in accommodating information newly brought to light (experiments are of necessity more difficult to devise) and from its usefulness as a framework for making sense of the interaction of physical and biological change on the surface of the Earth. The theory is value-free ("fittest" being a technical term) and appears to make sense even when things seem to be going backwards (as during mass extinctions). The generality of the theory is both its strength and its weakness.

Contradictory evidence is hard to find. It is, for example, robust enough to accommodate S.J. Gould's notions of punctuated equilibrium (which are not the death of darwinism). Even a modicum of lamarkian inheritance might exceptionally be tolerable. The canard (due to Popper) that evolution is not testable because it remains largely unquantitative is belied by the many cases in which evolutionary ideas have been a guide to the solution of many practical environmental problems in recent decades.

Creationists would not have made such headway in Louisiana if the truth about evolution had been better understood, and if the general opinion of the nature of science had been better informed. So much is illustrated by last week's dissenting opinion from Justice Antonin Scalia and Chief Justice William Rehnquist, who says that "we have no basis on the record to conclude that creation science need be anything other than a collection of scientific data supporting the theory that life appeared abruptly on Earth". Such an opinion is tenable only by those who believe there to be a legitimate theory for each selected subset of data, perhaps because they do not know (that is the charitable explanation) that generality matters and that the distinction between occasional discrepancies from theories and the usually telling contradictions that bring them crashing down are crystal clear. The schools of Louisiana plainly need help in grappling with these issues, but they are not alone.

Everybody should recognize that there are many adults who need help in reconciling evolution with their religious beliefs, although the conflict is not unavoidable. The creation scientists, some of whom have a scientific background, deserve no such sympathy. Not all of them are charlatans, but some are. More commonly, they are deeply mischievous people sustained only by the gullibility of those who believe that *Genesis* is correct, but who know little of what science is about. Even those who say that their objective is merely to make sense of some puzzling datum are mischievous when they decline to follow the criteria that other people readily accept for the validation of isolated data. But why mischievous? Because, as the Louisiana case has shown, the consequence is that adults are misled and children are badly taught. Especially when there is a crying need for a better understanding of what the world is like, this is a serious disservice to the rest of us. □

All change at work

In the long run, innovation does not destroy jobs but creates them.

THE belief that the improvement of technique spells the end of people's prosperity, widely entrenched since the luddites smashed textile machinery in nineteenth century England, has never taken hold in North America. Now (see p. 649), a panel of the US National Academies has done its best to ensure that it never will. The report last week of the Cyert committee should be a landmark in the battle against industrial obscurantism, if only because of the care with which it has gathered contemporary evidence about the interaction of innovation and employ-



ment — a field too long neglected by academics and public authorities alike.

The Cyert committee says many predictable but nonetheless sensible things. Let there be better training for those entering the labour market and, recognizing that change is bound to mean disruption, better arrangements for retraining those displaced from their jobs. It also gives a telling account of the process by which innovation (called invention) leads by development to widespread use. If the report is as widely read by politicians and even shareholders as it deserves to be, it may powerfully assist in taking the edge off the impatience with technology now commonplace. No doubt, some will complain that the arrangements suggested for assisting displaced workers would be the death of free enterprise but little is likely to happen quickly on that score.

For the rest, two features of the document stand out. First, it is a vivid reminder of the pace with which US investment in research and development is increasing. A three-fold increase by private industry in a quarter of a century is a remarkable achievement even by American standards, while the absolute value of this investment — now approximately \$50,000 million a year — is staggering, even by the standards of Japan. That federal spending on research and development has increased by a half in just a decade is similarly awesome.

So why is the United States so consistently disappointed in the outcome? Part of the answer is that there may not yet have been time for all this effort to work its way through to the marketplace. But many of the obstacles facing the United States in the exploitation of its inventiveness stem from the failure of the federal government to control its budget deficit and from the raft of troubles that has spawned. The Cyert committee, which makes clear its awareness of this problem, too timidly avoids a discussion of it. But is it wise to overlook the effects on competitiveness of the US government's need to borrow huge sums of money each year (more than \$220,000 million in the year to last October, no less than \$175,000 million this financial year), mostly from overseas? Until the closing months of last year, interest rates were as a consequence needlessly high, and a discouragement of innovation, while an overvalued dollar was a millstone around exporters' necks. Now the dollar has slumped, but interest rates have increased.

The present danger, underlined by the failure of the Venice summit two weeks ago to agree about anything that matters, is that economic activity in the United States, and world trade in general, will decline, setting in train a process by which federal deficits will rise, not fall, and in which real incomes will decline, most probably by inflation and by unemployment caused by economic forces. The Cyert committee's anti-luddite argument may be impeccable, but people thrown out of work by the mismanagement of the US economy will find little comfort in the knowledge that innovation has not brought them to the dole queues. □

Accelerator in doubt?

Do the new ceramic superconductors threaten the largest accelerator with obsolescence?

HIGH-energy physics is expensive, and the Superconducting Super Collider (SSC) one of its most expensive manifestations yet; the US government seems willing to stump up more than \$4,000 million for the project. No wonder that public arguments centre on the expense, not the science. Yet on both sides of the controversy, the arguments have become more strained as they have become further removed from the real question, that of why anyone should want to spend so much in the pursuit of physics of a particular variety. Now the controversy has been given a new twist by developments in a different field of physics — the discovery of the new ceramic superconductors.

In the argument, the most prominent obfuscation is the sup-

posed clash between Big Science and Little Science. Some opponents say that it would be better to spend the money on, for example, solid-state physics. But the hard fact is that some kinds of science are inherently more expensive than others; if solid-state physicists had good reason for spending money on such a scale, they too would deserve a hearing.

A variation on this theme is that building SSC will impoverish the rest of research; once there is a commitment to a big long-term project, the argument goes, the need to keep it moving will mean budget-trimming elsewhere when funds are short. But this line of reasoning, plausible though it may be, forgets that a government willing to spend money on Big Science is likely to be enthusiastic about science as a whole. Governments spend money on science on grounds that are a mixture of reason and whim; one kind of spending may or may not restrict another. People making a case for funds should set these imponderables aside and argue for their own projects on their own merits.

The second great undecidable issue about SSC is that of whether, or how much, basic research benefits technological industry. This is a messy issue, but SSC supporters must blame themselves for getting into the mess. They have extolled not only the bracing intellectual virtues of fundamental physics but also the sound practical merits of the associated engineering. The first point upsets many in other fields of research, who understandably resent being told that what they do is somehow less 'fundamental' than high-energy physics. The truth is that fundamentalism is a subject best left to theologians.

It is the second point, the supposed commercial spin-off of physics research, that has by an accident of timing tripped up SSC supporters. If there is a new range of superconducting materials around the corner, why hurry to build what could become the largest machine ever to be cooled by liquid helium? Why not instead make it a proving-ground for this new technology? Because the ceramic superconductors are (at least for the time being) mechanically brittle, chemically fragile, susceptible to magnetic fields and capable of carrying only small currents. Thin-film electronic devices may be on the horizon, but it will almost certainly be years before useful wires, let alone magnets, can be made. In any case, magnets are not the only expensive components of the SSC as now designed. Even if the optimists looking forward to stronger and thus smaller magnets are right, it might still cost \$3,000 million to build a working SSC. Those who say that SSC is a monstrous extravagance are unlikely to think it a bargain at two-thirds the price.

Yet although work on the new ceramics is moving so quickly that predictions are impossible, and although commercial applications are indeed many years away, we should know much sooner, perhaps in less than a year, what will eventually be possible. Here is both a difficulty and an opportunity for SSC supporters. If, on the draft timetable, niobium-titanium magnets are installed in the tunnel three or four years from now, and it is by then evident that a new kind of magnet could have been built, high-energy physicists will seem to be building an enormous steam engine just when the first diesel engine has been invented.

To avoid that danger, supporters of SSC, given to pooh-poohing the excitement about ceramic superconductors and thus at risk of seeming like stuffy reactionaries, should instead embrace the opposition and embark on a formal study of the practicality of the new materials. If, after six months or a year, it becomes plain that ceramics have indeed a long way to go, it would then be possible to renew the campaign for the SSC from a position of strength — that of having looked at all the alternatives and having chosen the best. If, on the other hand, study shows that a strong effort to use ceramics in magnets might succeed, high-energy physicists would be able to put their considerable weight behind that effort. Either way, SSC would be built, and high-energy physicists could continue to claim that they are a driving force behind technological industry. □

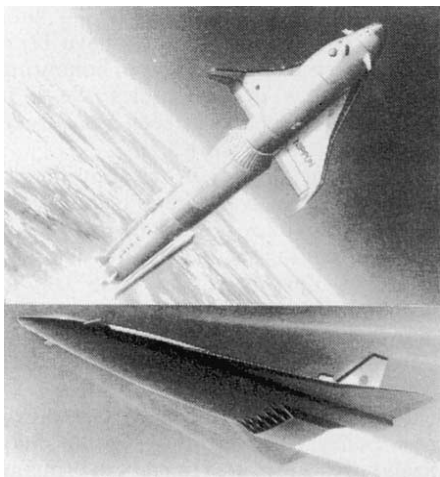
Japan plans space station in low Earth orbit

- National space budget may triple
- Not one, but two shuttles

Tokyo

JAPAN has new and optimistic plans to join the United States as a major space power. Two recently released reports see a Japanese space station in low-Earth orbit by 2010. The reports come from the long-term policy group of the Space Development Committee and the space plane advisory group of the Research and Development Bureau of the Science and Technology Agency. Both have been submitted to the Space Activities Commission, responsible for the nation's space policy, and will be used to draw up plans for 15 years, beginning in 1989.

Japan's present space programme is modest, with an annual budget of only ¥130,000 million (\$1,000 million). The



The two shuttles: one for cargo and one for passengers.

long-term policy group calls for a tripling in Japan's space budget. The US space station is central to the proposed programme up to the mid-1980s: ¥310,000 million is allotted to the Japanese experimental module for the station, ¥480,000 million for a co-orbiting platform and ¥120,000 million for an orbital manoeuvrable vehicle, or 'tugboat', to tow the platform to and from the station.

Design of the proposed co-orbiting platform and tugboat would begin in the late 1980s and early 1990s, respectively, and the policy group also calls for development of a polar-orbiting platform for low-Earth orbit at a cost of ¥180,000 million.

In the second half of the 1990s, the policy group advocates the development of a manned co-orbiting platform (¥480,000 million), a geostationary platform (¥360,000 million) and an inter-orbital transport system (¥900,000 million)

to link the latter with the space station. It is at this stage that the group foresees entry of Japan's private industry. It calls on the government to encourage private investment of about ¥3 million million through tax incentives and attractive government loans to develop, for example, a space factory for new materials.

The most ambitious plans of all, however, call for development of two types of Japanese shuttle costing over ¥2 million million and a Japanese space station for a further one million million yen. Development of the proposed station would not begin until 2000, and can be forgotten about for the moment. But some limited work on the shuttles has already started.

The space plane panel recommends development of an unmanned cargo shuttle called HOPE (H-2 orbiting plane) to be carried aloft by a modified version of the H-2 rocket, and an air-breathing space plane (like Britain's HOTOL) for manned flight. Two shuttles are advocated because of different priorities: safety is the prime concern for a manned spacecraft, while cost efficiency and large payload are required for a cargo plane.

The Science and Technology Agency's budget for this year includes ¥33 million for preliminary study of an air-breathing engine and the panel calls for joint development of the space plane by the agency's National Aerospace Laboratory, the National Space Development Agency, and the Institute of Space and Astronomical Science.

But will the Ministry of Finance be prepared to put up the funds? The six million million yen called for in the 15-year plan is not excessive when compared with projected outlays for space during the same period by the US and Europe (¥40 million million and ¥13 million million, respectively) and Japan's space budget has been lagging behind growth in the nation's gross national product. But an expenditure of ¥2 million million for shuttle development would be unprecedented.

So is Japan seriously intending to go it alone in building two shuttles? No, says Masahiro Kawasaki, deputy director general of the Research and Development Bureau of the Science and Technology Agency. Japan must commit herself to the development of new technology, he says, so that she can cooperate with other nations on an equal footing.

David Swinbanks

No go for the creationists

Washington

THE US Supreme Court has squashed an attempt by the state of Louisiana to require the teaching of "creation science" theory whenever evolution theory is taught. The court ruled that the Balanced Treatment Act violated the First Amendment to the Constitution which states that governments may not make laws "respecting an establishment of religion".

Although the 7-2 ruling applies specifically to Louisiana, it will be a model for all others, making it difficult if not impossible for states to require schools to teach the theory of "creation science".

The court had to decide whether the Louisiana legislature had a secular purpose when it passed the Balanced Treatment Act. Justice William Brennan, writing for the majority, agreed with the lower courts that had ruled that the law's intent was "to discredit evolution by counterbalancing its teaching with the teaching of creationism, a religious belief".

In a lengthy and spirited dissent, Justice Antonin Scalia argued that the state legislature did indeed have a secular purpose in passing the act. Justice Scalia argued that the act "did not fly through the Louisiana Legislature on wings of fundamentalist fervor". He noted that the parade of witnesses supporting the act presented "lengthy, and, to the layman, seemingly expert testimony on the origin of life". Failure to present evidence for the theory of "creation science" would deprive students of a balanced education.

But the majority ruled that arguments for the secular purpose of the act were a sham, only presented *post hoc*. Rather than enhancing academic freedom, said the majority, the Louisiana law undermines a comprehensive scientific education.

The Balanced Treatment Act was passed in 1981, but was challenged immediately in federal court, and never actually implemented. A similar law had been nullified in Arkansas in 1980.

Justice Scalia's dissent argued that there were issues of material fact to be determined in the decision about the Louisiana law, and that case should have been sent back to lower courts for a trial. Notably, a trial would serve the purpose of giving a definition to the term "creation science". Justice Scalia expressed astonishment that the court disbelieved Louisiana's secular intent, and attributed this to "an intellectual predisposition created by the facts and the legend of *Scopes v. State*", a case in 1927 based on a Tennessee law that tried to prohibit the teaching of evolution.

Joseph Palca

Britain losing out on its share of SDI research contracts

London

BRITAIN's participation in the US Strategic Defense Initiative (SDI) research programme has failed to produce the hoped-for lucrative contracts, the House of Commons Defence Select Committee says in a recent report.

When a memorandum of understanding about British involvement in research and development for SDI was signed in December 1985, there were projections that UK companies would win £1,500 million in contracts. The report says, however, that by March this year only \$34 million (about £20 million) worth of orders and commissions had been awarded; that is expected to rise to \$100 million by the end of the year.

"SDI participation may not be the great bonanza that some thought", the Ministry of Defence admitted in evidence to the select committee. The \$100 million total is only 1.56 per cent of the total SDI budget for 1986 and 1987. The committee notes that this is "disappointing".

More than 400 British companies have contacted the SDI Participation Office of the Ministry of Defence (MoD) expressing interest in the programme, but only 32 companies have so far been awarded contracts. About two-thirds of the funding so far is on a government-to-government basis, with most of this subcontracted to British industry, in accordance with Whitehall's aim of ensuring that not more than 15 per cent of the value of government-to-government contracts will be carried out in MoD research establishments.

The most valuable of these contracts in the "European Architecture Study", worth \$9.9 million, a "theoretical research study of the systems necessary for integrated defences of Europe within the context of a strategic defensive system for the defence of the continental USA and its allies against ballistic missiles".

Overall, the defence select committee concludes, "US funding of British SDI work has been directed much more at what former MoD chief scientific adviser Professor Sir Ronald Mason described as 'paper studies' such as the European Architecture Study, than at 'real system studies and innovative technological studies'. It must be a matter of doubt whether this situation will improve."

This predominance of "paper studies" militates against any appreciable levels of industrial spin-offs according to the report. Non-military spin-off would be much more likely to arise from more technological research, such as the European Eureka programme. Professor Mason told the committee: "If you have, say, in the Department of Trade and Industry,

clear ideas as to what technologies need sponsorship, then to do it through the back door of SDI seems to be a cock-eyed way of going about it."

Other European countries have also signed intergovernmental agreements to participate in SDI research, but most have fared even worse than Britain. Only West Germany has done better, with a total of \$42.5 million in SDI contracts by April this year. Israel had contracts worth \$10.8 million francs, \$8.1 million; Italy, \$5.1 million; Belgium, \$94,000; and the Netherlands, \$43,000.

The committee blames the poor performance of British and other non-US companies on "the intrinsic difficulties that face companies trying to penetrate the American defence market". As a major reason for continued involvement in SDI research, the committee cites the need to stay abreast of developments in strategic defence.

Kathy Johnston

Government science budget criticized

London

THE British government is failing to make available sufficient funds for scientific research and student support, a House of Commons all-party select committee has concluded. In a report published last week, the Education, Science and Arts Committee says that the existing volume of science research for 1987-88 and beyond "should at least be preserved".

Although expenditure on science is planned to increase in real terms from £527 million in 1986-87 to £558 million in 1987-88, there is a likelihood of real reductions in the following two years first to £548 million and then to £542 million. The committee dismissed government claims that the increased science budget in 1987-88 was serving to "maintain and enhance the strength and quality of the science base". It concludes that "the costs of science research will more than outweigh the extra resources. In short, the volume of science will be cut".

On student support, which is planned to decrease in real terms from £714 million in 1987-88 to £705 million in 1989-90, while student numbers increase, the committee urges the government to "find the resources necessary to start a period of gradual real increases in the value of student awards".

The report expresses disquiet about the lack of information on financing, administration and accountability of the new City Technology Colleges (see *Nature* 327, 359; 1987).

Simon Hadlington

Hungary a focus for astrophysics?

Balontfűred, Hungary

PLANS to develop Hungary as an international centre for astrophysics took a major step forward last week, says Dr Alexander Szalay of Budapest's Eötvös Loránd University. Szalay had just realized a long-standing dream — that of bringing leading astrophysicists of East and West together in Hungary — when his university co-hosted an International Astronomical Union (IAU) symposium on the structure of the Universe.

It is still rare for Soviet cosmologists to be able to travel to the West, so Hungary, with its liberal policy towards Western visitors, provides an ideal meeting place. At last week's symposium the Soviet Union sent a delegation of fifteen, about 10 per cent of all Soviet scholars specializing in cosmology, Szalay estimates. And as some members of the delegation were relatively young, there is perhaps a sign that the traditional Soviet approach to international trips is changing — previously only senior scientists have been allowed to travel, as a means of honouring them, but now, in astrophysics at least, it seems that travel may be seen as a normal part of a scientist's work.

Further evidence of a more liberal attitude in the Soviet Union is the recent visit of Dr Yakov Zel'dovich, doyen of Soviet cosmologists, to the United States. This was Zel'dovich's first US trip in decades. For many years Soviet astrophysicists have had to rely almost entirely on letters to maintain contact with US colleagues.

But even with Mr Mikhail Gorbachev's new policy of *glasnost* — openness — Szalay sees a role for Hungary in bringing East and West together. Government bureaucracies cannot change overnight, and it will still be easier for Soviet scientists to travel to Hungary than to the United States. Neither does Szalay see Hungary as merely a catalyst in the field of astrophysics. Hungarian astrophysicists believe that their contributions to the Vega probes of Venus and comet Halley fully justified their status as equal partners with the Soviet Union.

In keeping with his international outlook, Dr Szalay is also looking to the West for cooperative ventures. Negotiations are under way with the US National Science Foundation for a grant to finance exchange in theoretical astrophysics between Hungary and the United States. Szalay recognizes that the chances of East-West cooperation are better in theoretical fields than in observational astrophysics, where sensitive 'state-of-the-art' technology such as computer-controlled detectors may be involved.

Vera Rich

CNRS weakened by uncertainty but back in business

Paris

A SECTION of a bill passed in a late-night sitting of the Assemblée Nationale (the French lower house) on 12 June looks set to bring to an end a legal imbroglio that has paralysed the normal functioning of the main French research council, the Centre Nationale de la Recherche Scientifique (CNRS), for the past twelve months. The bill, due for Senate approval next week, will retrospectively validate the vital CNRS appointment committees declared unconstitutional last spring (*Nature* 324, 9; 1987).

High court rulings earlier this year ended the worries of many newly recruited researchers who had found their jobs under threat when the CNRS appointment committees — the Comité National — were suddenly dissolved. But uncertainties remained for new appointees in the life sciences (see *Nature* 326, 120; 1987). Measures in the new bill will remove this anomaly and allow promotions of senior scientists in all disciplines, also blocked by the dispute, to go ahead.

The root of the quarrel lay in Article 6 of a decree, drawn up under President Mitterrand's socialist government in 1982, that introduced a system of proportional representation in the election of Comité National members by CNRS staff. Back in 1983, the conservative Fédération des Syndicats Autonomes, the trade union representing medical lecturers and lawyers, questioned the legality of Article 6. But before the problem was finally resolved the socialist government found itself out of office.

The new right-wing government, elected in 1986, objected to what it saw as the excessive influence of the left-wing trade unions representing researchers. A little over half of the 990 members of the Comité National, which both appoints juries to select new recruits and decides on senior promotions and performance, is nominated by the unions representing CNRS researchers and university staff. Although the remainder of members are appointed by the government, union influence has been strong enough to oppose government reforms which would have reduced the separation of teaching and research and increased industry's responsibility for research.

When a right-wing trade union, the Syndicat Autonome d'Enseignants Médecine, managed in March 1986 to obtain a ruling by the High Court (Conseil d'Etat) declaring illegal the 1983 elections to the life sciences section of the Comité National, the new government saw its chance to act. The science minister, Alain Devaquet, abolished the entire com-

mittee, freezing new appointments and postponing the promotion of senior scientists.

A temporary measure gave two-thirds of the new recruits one-year contracts while the constitution of the Comité National was being amended. But the 285 scientists affected were not satisfied and formed a pressure group, the Collectif des Admissibles, to lobby for ratification of their posts and to join with unions to put their case before the High Court. Meanwhile Devaquet fell victim to his right-wing policies. When students took to the streets of Paris to protest against education reform he was forced to resign. A new minister, Jacques Valade, was appointed in January 1987. High Court rulings then gave both victory and defeat to the Collectif des Admissibles. The court ruled that Devaquet had acted illegally in abolishing the Comité National, thus opening the way for recruiting to proceed, but confirmed its earlier decision that elections in the life science section of the Comité National were invalid. Life scientists were thus left in limbo. Promotions of senior scientists were also in disarray because the Comité National had not completed its decisions before its demise.

Only after the Senate approves the new bill will everything return to normal (if time can be found for the bill before the summer recess in July). But the question still remains of why the mess was not sorted out earlier. According to Paul Janiaud, leader of one union representing researchers, the Syndicat National des Chercheurs Scientifiques, the government wished to exploit the chaos within CNRS to weaken opposition to reforms.

The government has cut grants to laboratories by 15–20 per cent, and changed the constitution of the CNRS to provide for increased representation of teachers in its electorate and to reduce the number of elected members of the Comité National. It also aims to abolish tenured posts for young researchers and to replace them with two- or three-year postdoctoral fellowships, a policy favoured by the director-general of the CNRS, Serge Feneuille. The CNRS will find it hard to recover from disruptions to its calendar. Appointments and promotions due to be decided in June 1986 will not be finalized until the autumn. Laboratory evaluations scheduled for October will be put back until next spring. In the meantime, CNRS has to elect a new Comité National and recruit new researchers. Many prospective candidates may have been dissuaded from seeking a career in CNRS and gone into industry — but this is exactly what the government would like. **Peter Coles**

Hitachi looks to UK

HITACHI, the Japanese electronics giant, is to create a computer software laboratory at its London headquarters to exploit the skills of the British in artificial intelligence. The programme, with an initial budget of more than £1 million, will be co-ordinated by Hitachi but at least two major contracts will be assigned, one to a university — Glasgow, Strathclyde and Edinburgh are being considered — and one commercially.

Despite having 10,000 computer programmers, Hitachi believes Britain has much of the world's expertise in artificial intelligence and that the British are better suited to writing programs for the non-Japanese user. **B.J.**

Sir Mark's warning

PROFESSOR Sir Mark Richmond, the new chairman of the Committee of Vice-chancellors and Principals of the Universities of the United Kingdom (CVCP), warned last



week that although government proposals to introduce a system of contract funding had positive aspects, if they were "driven very hard and in an unthinking way, everything gets into a straitjacket and there

is no room to innovate". In his inaugural speech, Sir Mark said that the cutbacks in higher education since 1981 had given the system "an enormous kick up the pants" and had reminded universities that society "does not owe us a living". He described as inevitable proposals to restructure the higher education system by concentrating high-spending research in centres of excellence, but added that if such a programme were put into practice aggressively the result would be "real problems of motivation and reorganization". **S.H.**

AMA on AIDS tests

THE American Medical Association, the largest medical organization in the United States, seems set to oppose compulsory testing for the HIV virus which causes AIDS and to back powerful new laws designed to protect the rights of those found to be seropositive for the virus. These recommendations are in a report being presented to AMA's annual meeting this week.

The report does not raise objection to the routine testing of immigrants, military personnel and prisoners in federal jails but it rejects Reagan administration plans to test individuals considering marriage and all hospital patients on the grounds that it would be a waste of resources and would cause distress to false positives. The answer is seen in voluntary testing, carried out in complete confidentiality, with counselling for those testing positive. **A.A.**

Acid rain — do shallow perceptions hinder progress?

London

EUROPEAN pressure on Britain to mend its ways on SO₂ emissions and vehicle exhausts could be alleviated by diplomacy, according to a report* issued this week by the British Policy Studies Institute and the Royal Institute of International Affairs. The report says that many of the difficulties over acid rain have arisen because of the shallow understanding by European states of each others' cultural and economic problems.

Much of the interest of the report bears on the long-standing disagreements between Britain and the Scandinavian countries about the degree to which atmospheric pollution from British sources damages recipient areas to the north-east. Britain and its public utilities, such as the Central Electricity Generating Board (CEGB), have been accused of dragging their feet on emission control.

Much of the report consists of an account of the internal factors that have determined the policies adopted in the affected European states, as follows:

Sweden. Affected by environmental damage to thousands of lakes, Sweden is disposed to give high priority to environmental issues. Its own SO₂ emissions are planned to fall by 65 per cent between 1980 and 1995. From later this year, all new vehicles will be required to comply with the tight US standards for NO_x emissions. The report says that Sweden, believing that East European countries will not be able to afford to reduce their emissions, is resigned to continuing pollution from them, but that it is far less charitable towards Britain.

West Germany. In the early 1980s, West Germany was stung into action by growing awareness of forest damage, considered an important part of the national heritage. The resulting policies are based on the view that the control of emissions can and should be afforded. But in spite of the installation of sulphur scrubbers and tax incentives for catalytic converters and unleaded motor fuel, significant reductions have not yet been achieved.

France. Because of its investment in nuclear power, France may benefit economically as other countries spend resources on reducing emissions. France is not regarded as a major source of damaging emissions but has undertaken to reduce emissions significantly.

Great Britain. While a significant contributor of sulphur depositions abroad, Britain is now requiring CEGB to install scrubbers on a scale sufficient to ensure continuing reduction of emissions. But the government resists a guarantee of faster progress on the grounds that there is insuffi-

cient evidence that Scandinavian catchments would recover so much more quickly that the cost would be justified.

The report notes that Britain is also exposed to political pressure from the European Communities, concerned not only about pollution but that there should be uniform standards affecting competition between European states.

The report suggests that there should be an attempt to make better known elsewhere the "considerable efforts" made in Britain to "safeguard environmental values". Specifically, it says that the Department of the Environment should be strengthened and that there should be fiscal incentives to persuade industry towards energy conservation.

Philip Campbell

• New estimates of trans-European sulphur and nitrogen budgets significantly increase the proportions of harmful depositions that can be attributed to source countries, particularly Britain. But

the new figures are unlikely to lead to a change in British policy which is more sensitive to estimations of ecosystem consequences.

The estimates come from the well-respected model of Anton Eliassen and colleagues at the Norwegian Meteorological Institute. Previous models of sulphur transport attributed about 14 per cent of depositions on southern Norway to Britain, but left about 50 per cent unattributed partly due to lengthy and circuitous airmass trajectories. The new model reduces the unattributable proportion considerably, identifying Britain as the source of 43 per cent of depositions with West Germany ranked second at 11 per cent.

The model has also been extended to account for nitrogen transport and deposition. Preliminary indications are that nitrogen oxides are transported at least as far as sulphur oxides and that the deposition differences of the two species mostly reflect differences in the geographical distribution of the respective sources. □

* *Acid Deposition and Vehicle Emissions: European Environmental Pressures on Britain*, by Peter Brackley. Energy paper 22 of the Joint Energy Programme, by Gower Publishing Company Ltd, Gower House, Croft Road, Aldershot, Hants GU11 3HR, UK.

UK participation in CERN is still hanging in the balance

London

AN international review of the European Organization for Nuclear Research (CERN) has failed to identify the level of budget savings that would guarantee Britain's continuing collaboration in the 14-nation venture. The review committee, set up at the request of Britain under the chairmanship of Professor Anatol Agram to consider the means and possible impact of reductions in the running costs of the laboratory, presented an interim report to the committee of CERN council last week. The 40-page document, which has not been published, criticizes the management of CERN, but emphasizes the excellence of the science.

The main recommendations of the review committee are a reduction in staff of between 10 and 15 per cent (400–600 people), commencing in 1988–89, and changes in the employment status of some permanent staff, to incorporate them into the French or Swiss national employment systems. A substantial reduction in the number of permanent contracts is called for, but no reduction of the science budget is recommended.

The immediate effect of the interim report has been agreement by the CERN council to limit the recruitment of new staff and to offer no new permanent positions, except where formal commitments have already been made.

In the meantime, a CERN management

team will be convened by the director-general to study the feasibility and consequences of the preliminary recommendations. The Agram committee will present its final report at the December meeting of CERN council, but it is unlikely that further significant action will be recommended.

The review committee expects savings approaching the 25 per cent by 1991–92 called for by a panel chaired by Sir John Kendrew, set up in 1985 at the request of the British government. Britain's Advisory Board for the Research Councils (ABRC) earlier this year recommended that the annual UK contribution should be around £30 million. But last year a sharp fall in the value of the pound against the Swiss franc meant Britain's contribution increased to £55 million, compared with £37.9 million in 1985–86.

ABRC's attitude towards continuing collaboration will depend on the precise level of savings attainable if Agram's recommendations are all adopted, and on the outcome of the Treasury's public expenditure survey in the autumn. Given that withdrawal from CERN requires notice of a full calendar year, it seems unlikely that Britain will wait for the review committee's final report. ABRC and the Science and Engineering Research Council will consider the preliminary recommendations next month.

Simon Hadlington

Academies say change brings expanding employment

Washington

RAPID technological change is not in itself a cause of unemployment, according to a report published last week by the US National Academies of Science and Engineering. On the contrary, says the panel under Richard Cyert, president of Carnegie-Mellon University, the displacement during recent years of up to a million skilled people a year from jobs in manufacturing industry may just as well be caused by the neglect of technology. But the panel argues for ways of softening the blow for displaced workers, some of which could be controversial.

The joint study, set up two years ago and supported financially by industrial companies and the chief federation of labour unions (AFLCIO), as well as from the academies' own funds, is likely to attract particular interest just now in the

tern". In any case, the effects of technological change on employment are more gradual than those of currency fluctuations, for example, while recent job losses in the United States are not caused by the rapid introduction of new technology, but by "increased US imports and sluggish exports".

The panel avoids full consideration of the changing pattern of international trade in relation to employment, citing its terms of reference, but notes that international technology transfer is no longer in one direction: "In many technologies, the United States no longer commands a significant lead over competitor nations".

While circumspect about international trade relations, the panel is unrestrained on US domestic policy. Among other things, it argues that the Job Training Partnership Act, which provides federal assistance for company schemes aimed at the retraining of workers, should be broadened and improved, at a cost that could exceed \$1,000 million a year at the present rate of job loss, and argues for "federal action" to ensure that workers displaced from their jobs are given at least 2-3 months' advance warning.

One member of the panel, economist Professor Anne O. Krueger (Duke University) dissents from mandatory warning

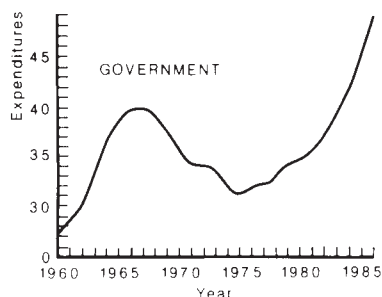
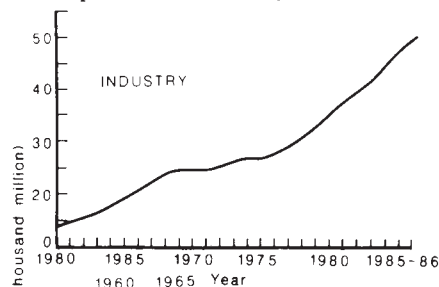
periods on the grounds that it would increase costs discourage companies from taking on workers and be tantamount to a tax on companies "already in difficulty". Much the same point is made by the Computer and Business Equipment Manufacturer's Association, one of the sponsors of the academies' study.

The panel takes a less gloomy view of the US educational system than many recent commentators, but complains that Black and Hispanic ethnic groups are neither participating nor succeeding as well as are whites and that, in any case, technological change probably requires more rapidly increasing attainment.

On the importance of research and development, the "sources of technological change", the panel says that federal defence research has become less important as a source of commercial innovation since the 1960s and that the point has probably now been reached when industrial companies' spending on civil research and development may be a more important source of military innovation than is defence research ("except in artificial intelligence and computer science") of commercial innovation.

Without implying cause and effect, the panel also points to the trough of research spending in the early 1970s, when the rate of growth of industrial spending was probably not great enough to make up for increased research costs and when federal research and development actually declined.

John Maddox



Research and development spending by US government and industry. In the 1970s government spending fell.

United States because of concern about the competitiveness of US industry and schemes for protecting US jobs by means of trade restrictions.

The essence of the panel's case is economic. According to Cyert, technological change means that less labour can create "higher quality products at less cost". While new technology may mean that some workers lose their jobs the "larger effect" is to reduce prices, to increase demand and to create jobs "in completely new areas".

Historically, the panel says, technological change has gone together with "expanding rather than contracting" employment and with increased earnings; the "future will see little change in this pat-

Australian scientists struggling to join the superconductor race

Sydney

THE wave of excitement generated in the world's physics community by the discovery of high-temperature superconductivity is sweeping through Australia too. It has not only affected physicists but has also put a gleam in the eyes of those who anticipate a rise in the value of Australia's abundant deposits of the rare earths from which the new superconductors are made.

Several groups have succeeded in producing superconductivity at 95 K, notably those at the Australian National University (ANU), University of New South Wales (UNSW), University of Western Australia, and the Applied Physics and Materials Science Divisions of the Commonwealth Scientific and Industrial Research Organisation.

The UNSW group was first off the mark and is the only one to have received any extra money for superconductor research — a grant of A\$500,000 from a fund administered by the Department of Industry, Trade and Commerce for research involving rare earths and ceramics. It is to be used by the UNSW team to develop

electronic devices in collaboration with Amalgamated Wireless of Australia. Applications are also being sought at ANU. Under development is a high-temperature superconducting infrared detector mimicking the superconducting edge bolometers at present used at liquid helium temperatures.

Most researchers are relying on whatever funds they were allocated before the breakthrough. Government grants are normally applied for 18 months in advance and speedy action seems unlikely. But the first steps are being taken to develop a strategy to bring together researchers, government and industry. Two hundred delegates from around Australia met at a symposium on the new materials and decided to establish a working group to guide research. Half of its 40 members will be researchers and half representatives of government and industry. Its first meeting will not take place until 16 July, which is late considering the frantic pace of research, but deliberately so in case the rules of the game change after the federal election on 11 July.

Charles Morgan

European biotechnology plans leave the Lords cold

London

PLANS by the European Commission for a substantial programme of biotechnology research have received only limited support from the UK House of Lords Select Committee on the European Communities. The conclusion of the committee's report, *Biotechnology in the Community* (HMSO, £9.30), published last week, endorses the British government's view that the proposed budget for the high-technology Framework research programme is too large. The report says the biotechnology programme "may be over ambitious and could duplicate activities supported elsewhere in the community". It recommends that "an appropriate appraisal procedure which limits the agro-industrial pilot programme only to projects which are likely to show promise of economic viability and recongised the possibilities of similar developments being carried out elsewhere should, however, be agreed and adopted".

The report will lend weight to Britain's lone veto of the £4,500 million, five-year Framework programme. Britain has been calling for a scaled-down programme of no more than £3,000 million, and has called current Community research projects "unfocused and poorly monitored". The Commission had been hoping that

Britain would agree to the research programme before the European Economic Community (EEC) summit in Brussels at the end of this month. Officials say that before the British general election, the government had been making "friendly noises", and the Belgians, who chair the EEC until the end of the month, are anxious to resolve the deadlock during their term.

Giving evidence before the Lords' committee, the government chemist, Dr R. Coleman, said that the Department of Trade and Industry (DTI) considered the Commission's proposal to spend 350 million ECU (European currency units; 1 ecu = £0.7) on agro-industrial development, agro-food research, agricultural research and biotechnology research was "significantly more than is justified". He suggested a figure of no more than 100 ECU for the whole package. The Ministry of Agriculture, Fisheries and Food proposed a limit of 20 million ECU for a biotechnology programme "if it contained significant industrial input".

The Agricultural and Food Research Council, which in 1985-86 spent £20.5 million on biotechnology research, is predictably enthusiastic about the programme and the proposed levels of funding.

Simon Hadlington

Cross-examination ends in TPA patent case

London

To the surprise of all involved, the cross-examination process in the tissue plasminogen activator (TPA) patent case in London's High Court (see *Nature* 327, 450 & 546; 1987) finished in less than two weeks. By the middle of this week, the closing arguments from the lawyers representing both Genentech, the US biotechnology company that holds British patents, and Wellcome, the UK pharmaceutical company that is challenging them, will have been heard.

Wellcome is trying to establish that the procedures used by Genentech's scientists to clone and express the DNA for TPA were 'obvious' at the time and therefore that the patents are invalid. Genentech, understandably, maintains that the techniques were not obvious in 1982. One of the problems at the time was how to isolate from a complex mixture of many different DNA molecules one that is present in very low abundance. Genentech scientists solved the problem for TPA by 'oligonucleotide hybridization'.

The obviousness of this and other steps in the procedure used by Genentech became the subject of close examination during the first two weeks of the case. The

broader picture was painted by the likes of Professors William Brammar of the University of Leicester and Paul Berg of Stanford University. More detailed testimony came from scientists involved in cloning TPA in various laboratories at the time.

The outcome of the case will be closely watched not only because of the ever-present interest in the fortunes of Genentech but because it is the first patent case involving recombinant DNA products to come to court. An added twist is that it concerns a substance that had already been isolated and, to some extent, characterized by conventional techniques. The question, therefore, is not so much whether a natural substance can be patented but whether its recombinant DNA forms and their production can be.

If Genentech wins the case the company less likely to have to fight the battle over again in too many countries, of which Japan may well be the first. If it loses it other than on a technicality of UK law, a series of battles seems certain.

Judgement is expected in mid-July. But if the judge needs more time to get to grips with molecular biology, the summer recess may delay a decision until October.

Peter Newmark

Awards out of the blue

Washington

THE first emotion experienced by winners of a MacArthur Fellowship seems to be disbelief. The reaction of Eric Lander, one of 32 winners of this year's awards* announced last week, is typical. Lander, a fellow at the Whitehead Institute and faculty member at Harvard University Graduate School of Business Administration, arrived home with his wife to find a message on his answering machine informing him he had won the fellowship worth \$205,000 over five years. Only after playing the tape a second time did Lander decide that the news might really be true.

The MacArthur Fellowship is unusual. Fellows neither apply for their awards, nor are they required to meet any performance standards to justify them. The programme is intended to give talented individuals an opportunity to be creative.

This is the sixth year of the awards programme. Some 100 nominators propose candidates to a 15-member selection committee, with final approval coming from the Foundation's Board of Directors. The size of the awards is based on the age of the recipient, plus an upward adjustment to take into account the newly taxable status of the award.

Winners have been chosen from a wide selection of academic and social interests. Superstring theorists seemed to have captured the imagination of this year's selection committee, with three of the four physicists winning awards working on strings.

Lander, whose research has been on mapping complex genetic traits, says he regards the award as "a very special pat on the back", adding that it will encourage him to "follow his nose" and not worry where his research will ultimately lead. Another winner, Ira Herskowitz, head of the division of genetics at the University of California, San Francisco, agrees the award is especially nice, because it rewards self-starters.

Joseph Palca

*Walter Abish, 55, writer; Robert Axelrod, 44, political scientist; Robert Coleman, 32, mathematician; Douglas Crase, 42, poet; Daniel Friedan, 38, physicist; David Gross, 46, physicist; Ira Herskowitz, 40, biologist; Irving Howe, 67, writer; Wesley Jacobs, Jr, 31, rural planner; Peter Jeffery, 33, musicologist; Horace Freeland Judson, 56, journalist; Stuart Kauffman, 47, biologist; Richard Kenney, 38, poet; Eric Lander, 30, mathematician; Michael Malin, 37, geologist; Deborah Meier, 56, school teacher; Arnaldo Momigliano, 78, historian; David Mumford, 50, mathematician; Tina Rosenberg, 27, journalist; David Rumelhart, 45, psychologist; Robert Sapolsky, 30, endocrinologist; Meyer Schapiro, 82, art historian; Jon Schwarz, 45, physicist; Jon Seger, 40, biologist; Stephen Shenker, 34, physicist; David Shulman, 38, philologist; Muriel Snowden, 70, community organizer; Mark Strand, 53, poet; May Swenson, 68, poet; Huynh Sanh Thong, 60, translator and editor; William Julius Wilson, 51, sociologist; Richard Wrangham, 38, ethologist.

IIASA appoints US director in hopes of attracting US funds

Laxenberg, Austria

HOPEFUL of a reinstatement of financial support from the United States, the council of the International Institute for Applied Systems Analysis (IIASA) has once again selected a US director, Robert Henry Pry, for the three years ahead. Pry was appointed at a meeting on 12 June by the IIASA governing council, which also appointed Vladimir Sergeevich Mikhalevich of the Soviet Union as chairman.

Pry is a former adjunct professor in materials science at the Massachusetts Institute of Technology (MIT) who is now a private management consultant. By appointing another US director, IIASA keeps links with the United States in spite of a National Security Council decision in March to block a \$500,000 grant from the US National Science Foundation (NSF) to the institute.

IIASA, a joint think-tank established in 1972 at Laxenberg, near Vienna, by the United States and the Soviet Union as well as ten other countries, has had a US director and a Soviet chairman since its inception. The institute brings in scientists from all the member countries, which now number sixteen, to study global problems such as forest decline, river management and world hunger. US funds were withdrawn in 1982 following a spy scandal and charges that IASA was leaking US technology to the Soviets. The NSF grant was the first attempt to restore US funding to IIASA since that time.

New chairman Mikhalevich, a mathematician specializing in systems analysis, is director of the 7,000-member Glushkov Institute of Cybernetics of the Ukrainian

Academy of Sciences. He is also a member of the Supreme Soviet.

The reasons for the US withdrawal of funds from IIASA were "not even political" at first, said outgoing director Lee. A victim of the 1981 Reagan budget cut, the \$2.3 million annual dues were reinstated later that year by Congress. But in 1982, the Reagan administration found "all kinds of reasons" to stop supporting IIASA. For instance, a Vax computer that had been installed in the 1970s was considered a technology leak to the Soviet Union. Although the deal was supported at the time by US politicians, buying a Vax was a "mistake", said IIASA secretary Jean-Pierre Ayrault.

Despite the conclusion of two National Academy of Sciences committees that there was no security problem at IIASA, US support was eliminated in 1982. Lee traces the cause to "a small group of powerful people" at the Department of Defense and elsewhere who believe that "any time you have something to do with the Russians you always lose". The 1987 National Security Council decision to block the NSF grant came despite approval of the grant by the US State Department.

Amid the political turmoil of the past five years, IIASA has shifted its focus to more application-orientated projects to try to attract external support. After 15 years, IIASA is seen to be entering a new phase in which it needs to justify itself scientifically. The choice of Pry as director reflects the hope that US policy towards IIASA may change in 1988.

Steven Dickman

A helping hand for inventors

London

A NOVEL scheme to minimize red tape and to help British inventors to exploit commercially the fruits of their research has been launched by a UK finance house in partnership with a US research group.

The British arm, called 3i (Investors in Industry), which claims to have provided finance and managerial support to dozens of start-up companies and currently has more than 60 investments in the United Kingdom and the United States, has joined forces with Research Corporation of Tucson, Arizona, to run the scheme. The British group has already created six companies spawned directly from university research while the US group claims to specialize "in converting academic inventions into commercially viable innovation and in technology transfer".

The new scheme, called 'Enterprise Cheques', will provide seed money for the development of "significant inventions and technological advances" of up to £25,000 for each project, in return for a stake in the technology being developed.

The programme, say the backers, is intended to appeal in particular to those in universities and research establishments but is also available to researchers conducting their own private work. All areas of interest in science and technology will be considered for the scheme which expects to issue about 15 cheques a year.

Managerial expertise is also to be made available to inventors when their projects reach the final stages of development. A new company will be created to exploit the respective technology or a licensing agreement devised so that others can exploit the development, whatever "is considered the best way forward".

According to 3i: "The UK is still a world leader when it comes to invention and the development of new technologies and ideas . . . We have designed the scheme to be as unbureaucratic and simple as possible".

Bill Johnstone

• University researchers were last year encouraged by the government to exploit their own inventions — rather than channel them through the bureaucracy of the British Technology Group (BTG). But ironically the BTG last week scored a major triumph in the defence of British patents and realized substantial royalties for the British inventors of advanced medical scanners. The US electrical conglomerate General Electric is to pay BTG an undisclosed sum, believed to be millions of dollars, in royalties accruing from the sale of scanners in the past two years. Among the beneficiaries will be teams from Nottingham and Aberdeen universities. □

Shake-up of UK junior ministers

London

IN a wide-ranging ministerial shake-up announced last week by the new Conservative government, the number of ministers in the Department of Education and Science (DES) increased from four to five, while at the Department of Trade and Industry (DTI), the title of Minister for Information Technology was dispensed with. The increased size of the ministerial team at DES is seen as a way to ensure a smooth passage of the Education Bill, which will introduce a major restructuring of higher and lower education. Mr George Walden, the former higher education minister who retired for personal reasons, is replaced by Mr Robert Jackson, who will continue Walden's review of student support, expected to be completed by the end of the year, and will preside over the finalization of the proposed system of con-

tract funding in universities and polytechnics. Baroness Hooper will take responsibility for the city technology colleges.

At DTI, the sacking of Mr Geoffrey Pattie, knighted in the Queen's Birthday Honours list, gives increased responsibility to his former number two, Mr John Butcher, who will take over DTI's research and development programme. Mr Kenneth Clarke, deputy to the new Secretary of State, Lord Young, will assume responsibility for European Community research and development. At the Department of the Environment, Mr William Waldegrave relinquishes the environmental portfolio to Lord Belstead, former Minister of State at the Ministry of Agriculture. Mr Michael Jopling, sacked Minister of Agriculture, is replaced by Mr John MacGregor, former Chief Secretary to the Treasury.

Simon Hadlington

South African boycott

SIR—The officers of the Linnean Society have met to discuss the matter raised in *Nature* (327, 273; 1987). We endorse the view of our council that you reported in that article, and reaffirm that we shall continue our policy of considering all submissions to our journals on scientific merit alone. We are taking steps to rectify the single isolated incident of rejection to which you drew attention and intend to ensure that in future the society's agreed policy is implemented.

W.G. CHALONER
(President)

*The Linnean Society of London,
Burlington House, Piccadilly,
London W1V 0LQ, UK*

Turin Shroud

SIR—I can assure Denis Dutton (*Nature* 327, 10; 1987) that all the participants in the workshop on "Radiocarbon Dating of the Turin Shroud" are acutely aware that the operation must be completely credible.

The workshop at Turin from 29 September to 1 October last year, chaired by Professor Carlos Chagas in his capacity as president of the Pontifical Academy of Sciences, involved representatives of the seven laboratories that will make the measurements, the British Museum, the Archbishopric of Turin and a representative of the Abegg-Stiftung in Bern, who will remove the sample from the shroud.

I presented the conclusions and procedural steps agreed to at the workshop as a poster at the International Symposium on Accelerator Mass Spectrometry at Niagara-on-the-Lake, Ontario, on 27–30 April. It will be followed by a paper to appear in *Nuclear Instruments and Methods*.

The procedures recommended are clearcut and straightforward. Although the testing laboratories will follow blind carbon-dating procedures, there will be no possibility of "tampering" with the shroud samples except as a result of collusion by a number of organizations including the British Museum, the Pontifical Academy of Sciences and the Archbishopric of Turin. The removal of the shroud sample by a noted textile expert from the Abegg-Stiftung in Bern, Switzerland, will be witnessed by representatives of the seven carbon-dating laboratories. A representative of the Pontifical Academy, the British Museum and the Archbishopric of Turin will supervise the shroud samples from their removal to their delivery, together with a dummy sample and control samples, to each representative of the seven laboratories. Equally careful procedures will attend the final analysis of the results from the seven laboratories. Six of the seven laboratories

have already participated in blind inter-laboratory comparison measurements supervised by the British Museum.

It is clearly important that the most significant scientific test on the Shroud of Turin, radiocarbon dating of the cloth, should be carried out in a manner that will convince people like Dutton that the results, whatever they may be, are believable. The only interest of the participating carbon-dating laboratories in "confidentiality" is that they be able to carry out the measurements under reasonably serene conditions.

HARRY E. GOVE

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Scientific fraud

SIR—Walter Stewart and Ned Feder (*Nature* 325, 207–214; 1987) give the results of a detailed analysis of 109 publications written by Dr John Darsee over a period of about three years. Their stated objective was to throw light on the vigilance of referees, editors of journals and Darsee's co-authors in meeting the standards conventionally accepted as necessary in the scientific literature.

Having catalogued all the errors and lapses of normal standard that they can find in these publications, the authors imply that these errors could have been avoided if the co-authors, editors and referees had been sufficiently vigilant. Unfortunately this is often not true, as their own paper shows. One of their criticisms of one of Darsee's papers is: "The summary of the paper gives the urinary taurine levels of the family members with congestive cardiomyopathy as ranging from 411 to 536 mg taurine per g creatinine, but the text and Table 1 of the same paper give for the same measurement 426 ± 45 mg taurine per g creatinine (mean $\pm$ standard deviation). Simple inspection suggests that these two sets cannot simultaneously be valid." This is described as a "major error which is fundamental and call[s] into question the validity of the paper's main conclusions. More typically, however, the errors simply reflect on the care with which the paper had been prepared." Unfortunately, the authors are wrong as simple inspection does not suggest the conflict between these data items. In fact, if we use the admittedly extreme data values of 410.51, 410.51, 410.51, 410.51, 410.51, 410.51, 410.51 and 536.49, we get (to the nearest integers) a range of 411 to 536 and a mean $\pm$ s.d. of 426 ± 45 .

This example raises two immediate questions. The most obvious is that it brings into question the authors' detailed count of errors and lapses from the 109 publications that they reviewed. More important, however, is that it brings into question their assumption that this type of

error can be avoided if suitable care is taken by editors, reviewers and the co-authors of publications. As the editors discuss in *Nature* (325, 181; 1987) and Barbara Culliton discusses in *Science* (235, 422; 1987), this paper has been under extraordinary study for over three years, but it still contains an error that the authors would classify as a type 1 lapse, "explainable simply by carelessness of excessive haste".

If the results of the Stewart and Feder paper are to be used, the listing of the errors and lapses that have been found by them must be independently audited by outside reviewers, as many of the small number that they have released have been questioned. Eugene Braunwald (*Nature* 325, 215–216; 1987) analyses many suggested errors or lapses, and also states that Stewart and Feder have refused to release the specifics and location of their purported errors from Darsee's papers. Without this release and a subsequent independent analysis (particularly by Darsee's co-authors), I cannot see how their detailed data can be used.

J. DENBIGH STARKEY

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Crystal clear

SIR—I should like to comment on the critical review by J. W. Emsley (*Nature* 323, 377; 1986) of our new publication, *Molecular Crystals and Liquid Crystals Bulletin*, and of our decision to issue separately *MCLC Letters*.

The criticism neglected to mention that *MCLC Bulletin* is distributed at no cost to all subscribers to the parent journal. It also does not make it clear that we have had a Letters section for some time, and simply decided to make it available as a separate optional subscription item rather than an obligatory part of a subscription to the parent journal. The parent journal *MCLC* appears monthly and contains papers dealing with liquid crystals, low-dimensional solids, molecular crystals, and (being added in 1987) nonlinear optical phenomena. The journal was established in 1966, and periodically introduces new services. Free services hardly deserve to be judged by the same criteria one applies to journals. Creating options should not be judged as an attempt at price escalation.

MARTIN B. GORDON

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Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only. □

Don't laugh at Aristotle

There has been an amusing demonstration that heavy objects may fall to the ground faster than lighter objects, but there is not yet much of a case for resuscitating Aristotle's physics.

THE late Leon Rosenfeld, mostly a mild-mannered man, used to come nearest to rage on the subject of Aristotle, whom he would accuse of having single-handedly held back science for the best part of 2,000 years. Aristotle's physics, once turned into general orthodoxy, was especially malevolent because it was at once ingenious and intuitive. What more clever than the notion that bodies are sustained in motion by the force of the air rushing in to fill the space they vacate? And what more natural than to suppose that massive objects will fall more quickly towards the Earth than those that happen to be lighter?

Now, of course, all that has changed. Everybody knows that the pre-galileans were misled by the buoyancy and friction of the atmosphere, but — in case there should be surviving doubts — there are still a number of elementary schools at which teachers put a coin and a feather in a glass tube, evacuate the air and demonstrate that feathers can also drop like stones. That is why two physicists from the University of Massachusetts at Amherst, John F. Donoghue and Barry R. Holstein, are now entitled to a modest joke by giving a recent paper the title "Aristotle was right: heavier objects fall faster" (*Eur. J. Phys.* **8**, 105; 1987). The effect, it will be appreciated, is only small.

In reality, this is not the first recent occasion on which a revision of the galilean opinion might have been called for. The possibility of a short-range force between massive objects dependent on the specific baryon number of the materials concerned, suggested last year by a re-examination of data collected by Eötvös in the early 1920s, would have made the acceleration of objects towards the Earth dependent on chemical composition. (Apparently, it remains to be seen whether that conclusion is confirmed.)

Donoghue and Holstein do not pretend to have found a new phenomenon, but merely to have calculated the consequences of the interaction between objects and the surrounding vacuum at temperatures other than absolute zero. Their account in the *European Journal of Physics* raises a number of intriguing issues.

The notion that the mass of an object may be a function of its temperature, and of that of the surroundings, is one of the oddest in spite of having been canvassed in the 1930s by R. C. Tolman in his book

Relativity, Thermodynamics and Cosmology but on the basis of largely formal arguments in thermodynamics. The explanation now given has the virtue of making explicit a mechanism by which such an effect might arise.

But which mass? The starting-point is inevitably the formal distinction between inertial mass (the quantity in Newton's second law of motion) and gravitational mass (which appears in Newton's law of gravitation). Newton was the first to appreciate that the two masses must be identical, which also follows from the principle of equivalence, the doctrine that the laws of physics should appear the same in frames of reference moving uniformly relative to each other, the essence of which was appreciated long before the special theory of relativity. As it emerges from what Donoghue and Holstein say, it is the inertial mass that varies with temperature.

Brooding about the nature of the electron mass towards the end of the nineteenth century remains a pointer to the contemporary argument, at least in the sense of H.A. Lorentz's attempt to identify some part of the mass of a point charge with the energy of its constituent matter in its own electrostatic field; the result, numerically infinity, was a harbinger of the difficulties field theorists now resolve (or rationalize) by their technique called renormalization.

In the modern version of this argument, the vacuum is capable of supporting radiation states (materialized as photons), each of which may in principle be excited at any time, subject only to the amount of energy and momentum that is available. Indeed, at temperatures other than absolute zero, the vacuum will be filled with black-body radiation of precisely the kind defined by Planck. But, even at absolute zero, the proper calculation of the state of an isolated charge in a box requires the consideration of all possible states of the system in which photons arise spontaneously and then, to ensure conservation of energy and momentum, disappear again, again spontaneously (whence the name "virtual").

Donoghue and Holstein have several things to say, not the least interesting of which is that the expression for the energy of the system known in dynamics as the hamiltonian represents what is called the free energy in thermodynamics, and is to be identified with the inertial mass of the

object, that the gravitational mass is derived from what is called the energy-momentum tensor in general relativity and that the difference between the two energies (or masses) is, in thermodynamics, the product of the temperature and entropy. This boils down to the assertion that the difference between gravitational and inertial mass will be the product of temperature and the rate of change of inertial mass with temperature (other variables being constant).

By good fortune, the simplified calculations show that, even though the presence of the vacuum influences both masses at absolute zero, the effects are identical so that the principle of equivalence remains valid. Although this is not necessarily a relativistic problem, it turns out to take account of relativistic virtual states, those in which photons create fleeting electron pairs, for example.

At temperatures other than absolute zero, this simple cancellation of effects does not apply. Indeed, given that the inertial and gravitational masses differ by quantities related only to the dependence of inertial mass on temperature, it is inevitable that the two quantities should differ, and by a quantity proportional to the square of the temperature (in degrees K). The gravitational mass is always less than the inertial mass. The gravitational acceleration of a charged particle near the surface of the Earth would differ from that at absolute zero by a fraction proportional to $(T/M)^2$, where T is temperature and M mass. This is the origin of Aristotle's rehabilitation; the greater the mass of an object, the less its gravitational acceleration appears to be diminished.

That at least is what the authors say. But the effect is very small; for an electron at 300 K, the fractional departure of gravitational acceleration from that expected at absolute zero comes out at 3 parts in 10^{17} — some five orders of magnitude beyond what is measurable. For nucleons, whose masses are greater, the effect will be smaller, as will be that for real matter. No doubt the authors would say that the coupling of nuclear matter to other than the electromagnetic field, potentially capable of yielding similar effects, would have even smaller consequences.

The upshot, then, is not so much a vindication of Aristotle's position, but what might be called a dismissive demonstration that he was wrong from the start.

John Maddox

Palaeoanthropology

Evolution and palaeobiology of robust *Australopithecus*

Eric Delson

THE evolutionary lineage that ends with living humans can now be traced more accurately back towards 4 Myr (million years) ago, partly because of studies of a second major early hominid clade (evolutionary radiation or sub-lineage), the 'robust' species of *Australopithecus*. New fossils relevant to this group were presented at a recent symposium*, but more important than these were re-analyses of known data which led researchers closer to a general agreement on the early phases of human phylogeny. Several previously suggested evolutionary trees were widely rejected in favour of a new interpretation which followed from the description by R. Leakey and colleagues last year (see my News and Views article, *Nature* 322, 496–497; 1986) of an ancient robust skull.

As many as six African species that lived 4–1 Myr ago are now placed into the broadly defined genus *Australopithecus*, presumably including the ancestry of *Homo* as well as the distinctive robust group. It is the relationships among these species (especially the latter clade) and their palaeobiology which the meeting was designed to clarify.

A carefully defined chronological framework is required to answer questions about the number of contemporaneous species and to help to allocate fossils to these species. Frank Brown (University of Utah) presented such a framework for the Lake Turkana Basin site units that have yielded most early hominids from East Africa. The find-spot of each major specimen was relocated and tied into a revised stratigraphical column, resulting in temporal placements with narrow error of margins only ± 0.05 – 0.1 Myr. In addition, Brown's palaeogeographical studies demonstrate the

presence of a lake in the basin before about 3.5 and after 2.0 Myr ago, but not certainly in between. The lack of deposition in the Koobi Fora region from 2.5–2.0 Myr ago was probably caused by volcanic uplift in the south-east segment of the basin, rather than caused by any climatically linked phenomena.

Recently published data indicate that *Australopithecus afarensis* ranged between 3.5 (Laetoli) and 3.1 Myr ago (Hadar); biochronology in southern Africa cannot decipher the relative age of the *A. robustus* sites Swartkrans (member 1) and Kromdraai B, but both appear to be roughly 1.9–1.6 Myr old (Elizabeth Vrba, Yale University; my own work). Swartkrans can be divided into five members, of which the lower three each yield specimens of robust *Australopithecus* and *Homo*, as well as stone and bone artefacts. These accumulations may have each taken only 10–20,000 yr to form if the Holocene, warm-climate member 5 is a valid guide, but whether they together span 0.1 Myr or over 1 Myr was debated (Bob Brain, Transvaal Museum, Pretoria). New dental specimens reveal no trends across this time interval (Frederick Grine, State University of New York at Stony Brook), but the postcrania suggest that *Australopithecus robustus* was physically capable of making the tools recovered (Randall Susman, Stony Brook).

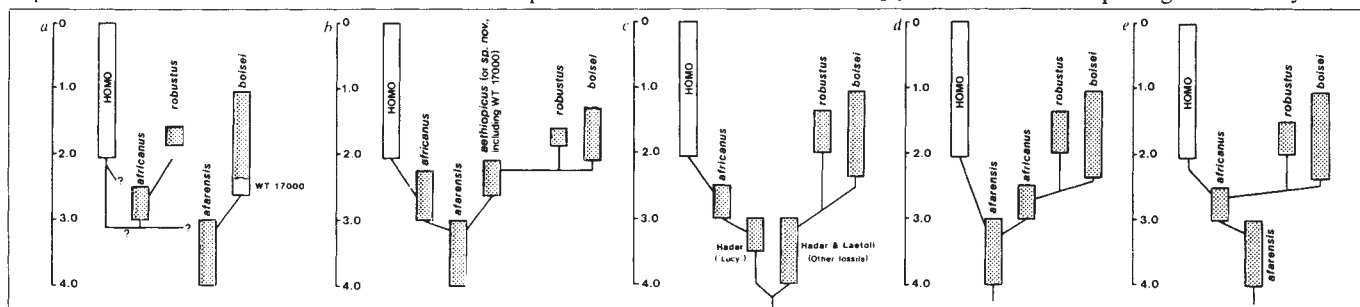
New fossils from the West Turkana region of Kenya support last year's find of WT 17000, a distinctive robust cranium slightly younger than tuff D (2.5 Myr ago). Alan Walker (Johns Hopkins University, with Richard Leakey, National Museums of Kenya) continued to argue that these fossils graded into the younger *A. boisei* known from 2.1–1.5 Myr ago and that such an evolving lineage should not arbitrarily be divided into species. He also presented fossils that convincingly

supported the allocation of WT 17000 and allies to a population previously known from the fragmentary toothless mandible Omo 18-1967-18 (2.6 Myr ago). He rejected the inference of large anterior teeth in WT 17000 as a functional correlate of the posterior placement of the sagittal crest and posterior temporalis muscles, but most participants disagreed with his interpretation. Donald Johanson, William Kimbel (Institute of Human Origins, Berkeley) and Tim White (University of California, Berkeley) argued for recognition of this early robust group as *A. aethiopicus*, a name given formally to the Omo mandible in 1978 by Arambourg and Coppens. I tentatively accept this view and will use the name here, although others might object.

Kimbel and White discussed variation in *Australopithecus* samples, suggesting that the Sterkfontein collection of *A. africanus* is among the most variable, whereas that from Hadar and Laetoli combined is more homogeneous. A new face, palate and partial vault from Sterkfontein presented by Ronald Clarke (Wokingham, United Kingdom) strikingly supports that view, but there was little acceptance of Clarke's claim that his Stw 252 (and STS 71, among others) represents a new species tending towards the robusts. East African dental evidence does reveal that lower premolars older than about 2.1 Myr ago (*A. aethiopicus*?) shared conservative features with Swartkrans fossils and could be distinguished from more derived younger specimens of *A. boisei*, while a third dental morph occurs at Omo between about 2.8–2.2 Myr ago, possibly a 'gracile' taxon leading into *H. habilis* (Gen Suwa, University of California, Berkeley).

A. boisei was also shown to be distinct in the ultrastructure of the molar enamel, which was both much thicker and somewhat differently built up than that of other hominoids: more secretory cells were active at one time, to lay down a thick enamel layer rapidly, with less emphasis on stress resistance (Lawrence Martin and Grine, Stony Brook; Bernard Wood, University of Liverpool). In terms of body-size estimates for these species, there was surprising uniformity both

*The Evolutionary History of the Robust *Australopithecines*. State University of New York at Stony Brook, 27 March–1 April 1987.



Phylogenetic schemes for *Australopithecus* species preferred by: a, Walker and Johanson; b, Delson, Grine, Howell, Olson; c, Olson's 1981–85 scheme, no longer adopted by him; d, Johanson and White's 1979–85 scheme, no longer supported by them; e, Skelton, McHenry and Drawhorn, 1985. Scheme b is now the most generally accepted. (Courtesy of F. Grine.)

across taxa and between workers (William Jungers, Stony Brook; Henry McHenry, University of California, Davis). Using an ape model based on limb joint size (but excluding modern humans), Jungers estimated average sample weights as follows: *A. afarensis*, 58 kg; *A. africanus*, 53 kg; *A. robustus*, 62 kg; and *A. boisei*, 63 kg. The Hadar range of 30–81 kg included all but the largest (88 kg) robust individuals.

There are now only two main hypotheses about the phylogenetic relationships among the early hominid species, both of which seem to agree that *A. afarensis* was a conservative species near the common ancestry of all later forms. The first hypothesis (*a* in the figure) is that *A. boisei* includes *A. aethiopicus*, *A. africanus* led only to *A. robustus* and the ancestry of *Homo* is unclear (Walker, Johanson). The second (*b* in the figure) is that *A. aethiopicus* is the sister-taxon (or perhaps common ancestor) of the later robusts, whereas *A. africanus* may be the sister of *Homo* (Delson; Grine; F. C. Howell, University of California, Berkeley; Kimbel; Todd Olson, City University of New York Medical School). The previous hypotheses that *A. afarensis* actually included two species, a robust taxon leading to later forms (effectively via *A. aethiopicus*) and a 'gracile' form including 'Lucy' which with *A. africanus* was on the *Homo* line (*c* in the figure); or that *A. africanus* was on the robust clade with *A. afarensis* leading directly to *Homo* by way of an unknown intermediate (*d* in the figure); or that *A. africanus* was a (post-

afarensis) common ancestor of the robusts and *Homo* (*e* in the figure), were generally rejected by their original proponents because of the conservative features shared by *A. aethiopicus* and *A. afarensis* to the exclusion of *A. africanus*.

Participants broadly rejected the first hypothesis, implying polyphyly of the robust species, because of the many derived features shared by *A. robustus* and *A. boisei*. Only one or two derived features are shared by *A. aethiopicus* and either *A. robustus* or *A. boisei*, although the three species do share a group of character complexes uniting them in the robust clade.

Thus, the second hypothesis is the least contested, but is by no means universally accepted. If a strictly cladistic classificatory scheme were to be followed, as argued by Grine and Olson, among others, *A. africanus* could be included in *Homo*, with the robust species termed *Paranthropus* and the genus *Praeanthropus* resuscitated for *A. afarensis*. However, most workers prefer to retain a more broadly defined *Australopithecus* pending detailed analysis of the new fossils and clearer determination of the phyletic role of *A. africanus*, which remains pivotal after more than 60 years of controversy. □

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Microbiology

Synthesizing designer drugs

Chris Higgins

THE rapid spread of antibiotic-resistance determinants in pathogenic bacteria gives an urgency to the development of new antibiotics. There is no doubt that many natural antibiotics remain to be isolated from *Streptomyces* and other bacterial or fungal species. Alternatively, novel antibiotics can be specifically designed and synthesized. Such synthetic antibiotics have the potential advantage that, as they cannot previously have been encountered by the target organism, resistance determinants are unlikely to be present in the population. On page 730 of this issue¹ a paper by Hammond and colleagues describes a new class of synthetic antibiotics. This paper provides an elegant illustration of the rational approach to designing novel antibiotics. Furthermore, the principles exploited in this paper are not only applicable to the design of other antimicrobial agents but may also find a use in the design of herbicides, chemotherapeutic agents and other drugs.

When designing a new antimicrobial agent, two criteria must be met: the antibiotic must be directed against a target that is specific to the organism under attack; and it must be able to gain access to that target. The latter criterion is particularly important as biological membranes are essentially impermeable to hydrophilic molecules and, unless the antibiotic is directed against an extracellular target (of which there are rather few), it must be able to gain entry to the cytoplasm via one of the complement of specific permeases in the cell. As most permeases are relatively specific, this is not always an easy problem to overcome.

Hammond and colleagues describe¹ a series of antibiotics directed against the synthesis of lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria. LPS is essential for pathogenicity and the viability of these bacteria in their natural environment, and is unique to this class of

organism. Thus, the enzymes involved in LPS biosynthesis provide one of the most promising targets for the design of selective antibiotics against Gram-negative bacteria. Indeed, it is perhaps surprising that this potential target has not been fully exploited before. Several analogues of LPS biosynthetic intermediates were synthesized and, as expected, some were potent, specific *in vitro* inhibitors of one of the key enzymes of LPS biosynthesis. But when tested *in vivo*, these analogues lacked significant antibacterial activity. An old problem had reared its ugly head; these highly specific inhibitors were unable to gain access to the target enzyme in the cytoplasm and, consequently, were not toxic to growing cells. To overcome this problem, the authors adopted the 'Trojan horse' approach: the toxic moiety was attached to a carrier molecule such that it became a substrate for a known permease and could gain 'illicit' access to its cytoplasmic target. The carrier selected was a small peptide. When the toxic warhead (abbreviated to NHdKDO) was linked to the carboxyl group of dialanine it became a potent bacteriocidal agent, active against a wide spectrum of Gram-negative bacteria but not against Gram-positive species.

Several years ago, the groups of Ames² and of Gilvarg³ independently showed that peptides can be used to fool the cell into taking up molecules that otherwise cannot enter the cytoplasm. Peptides are ideal carriers because of the rather unusual specificity of bacterial peptide transport systems⁴. That is, affinity for these transport systems requires the peptide to possess a protonated amino-terminal group and an unmodified α -peptide bond, but is essentially independent of the nature of the amino-acid side chains and of the carboxy-terminal carboxyl group. Biologically, this specificity is hardly surprising. The cell could not possibly have a separate transport system for each of the 400 dipeptides and 8,000 tripeptides that could potentially be encountered in protein digests, and it makes eminent sense to evolve a single transport system that recognizes those structural features common to all peptides. This fact can be exploited such that a normally non-permeant molecule can gain access to the cytoplasm if attached to the carboxyl group of a peptide or if it replaces one of the amino-acid side chains.

Nature, of course, was first to exploit this idea: several natural antibiotics, such as bialaphos secreted by *Streptomyces*⁵ or bacilysin produced by certain *Bacillus* strains⁶, have the toxic moiety attached to a small peptide. The peptides enter the target cell via the peptide transport systems, are cleaved by one of the battery of intracellular peptidases, and the toxin is released free in the cytoplasm. One further relevant point is that *Escherichia*

coli and *Salmonella typhimurium* actually have three peptide permeases with different but overlapping specificities^{7,8}. It is important when designing any peptide antibiotic that it enters the cell by more than one of these permeases such that at least two independent mutational events will be needed to develop resistance: this is, fortunately, the case for the new class of NHdKDO-peptide antibiotics.

Peptide transport systems of similar specificity are widespread, not only in microorganisms but also in plants⁹ and many mammalian tissues¹⁰. Thus, there are endless possibilities for designer herbicides and chemotherapeutic drugs. Finally, as was originally pointed out by Ames *et al.*², illicit transport via peptide

transport systems can be an invaluable experimental tool, allowing circumvention of the permeability barrier and direction of otherwise impermeable molecules into the cytoplasm. □

1. Hammond, S.M. *et al.* *Nature* **317**, 730 (1987).
2. Ames, B.N. *et al.* *Proc. natn. Acad. Sci. U.S.A.* **70**, 456 (1973).
3. Fickel, T.E. & Gilvarg, C. *Nature* **241**, 161 (1973).
4. Payne, J.W. *Microorganisms and Nitrogen Sources* (Wiley, Chichester, 1980).
5. Murakami, T. *et al.* *Molec. gen. Genet.* **205**, 42 (1986).
6. Kenig, M. & Abraham, J.J. *gen. Microbiol.* **94**, 37 (1976).
7. Higgins, C.F. & Gibson, M.M. *Meth. Enzym.* **125**, 365 (1984).
8. Hiles, I.D. *et al.* *J. molec. Biol.* **195**, 125 (1987).
9. Higgins, C.F. & Payne, J.W. *Planta* **138**, 217 (1978).
10. Matthews, D.M. *Physiol. Rev.* **55**, 537 (1976).

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Oceanography

Dense water off continents

John A. Whitehead

BOTTOM water is the coldest and hence the most dense water in the ocean. It starts as surface water in polar regions such as the Greenland-Norwegian Sea, the Weddell Sea in Antarctica or the Labrador Sea, and sinks as it cools. But what are the specific details of the journey? A similar situation is found in inverse estuaries where evaporation causes water to be more saline and dense than the offshore sea water. Thus at the mouth of the estuary, sea water flows in over the dense water, which exits onto the continental shelf as a gravity current. Lennon and co-workers report on page 695 of this issue¹ an outstandingly detailed study of such a process in Spencer Gulf in Australia. The lessons they learn can usefully be applied to the formation of bottom water.

There are two principal processes that convey cold, dense water in the polar regions from the surface to great depth. Both processes are difficult to observe directly because they occur only in a few inaccessible regions and often only at the height of winter. The first involves deep-convection events in which convection suddenly (over days to weeks) penetrates to great depth in deep oceanic basins at the coldest time. Such events have been observed only two or three times. The second process is the accumulation of extremely cold, salty and therefore dense water on the shallow continental shelves on the edge of the polar oceans. These dense waters migrate to the shelf break and flow down as shelf-break gravity currents, possibly channelled by submarine canyons.

This second process is thought to be particularly important in the formation of Antarctic bottom water, which is the most voluminous ocean bottom water. Water near, or on, the shelf of the Weddell Sea

off the coast of Antarctica could form Antarctic bottom water. No observations have been made of the gravity current that would convey water from the shelf of the Weddell Sea to the deep ocean. Indirect evidence is found² in the form of frontal structures near the shelf break. The flow of water implied by the front is towards the western end of the shelf. This region is permanently covered by ice and is inappropriate for an oceanographic survey in any season, so the gravity current might never be observed directly.

Fortunately, dense water elsewhere accumulates on shelves and migrates to the shelf break for other reasons, often on a smaller scale. The work of Lennon and colleagues in this issue¹ is on one such system in Spencer Gulf, South Australia, where water is driven to high salinity by evaporation during the summer. In the early autumn, the water cools, sinks and spreads out along the bottom. In so doing it goes from the gulf onto the continental shelf and draws fresher water into Spencer Gulf from offshore. Once free of the confines of the gulf, the current of saline water veers to the left, possibly assisted by the Earth's rotation, and intersects the continental-shelf depth contours as a gravity current. Lennon and colleagues traced it all the way to the shelf break where it cascades off the shelf in the region of Du Covedic canyon.

Similar systems have been observed elsewhere. Dense water is formed in Bass Strait, South Australia³ in winter and follows the edge of the continental shelf in a narrow north-flowing stream creating high-salinity intrusions at 200- to 800-m depth in the Tasman Sea. On the shelf at the northern end of the Adriatic Sea, dense water formation was observed⁴ in the winter of 1961. This water is said⁵ to be

the main source of the abyssal water of the eastern-Mediterranean sea. A similar situation occurs in the Gulf of Lyons⁶, but the water there sinks to only 350 m and so does not contribute to deep water in the western-mediterranean basin.

There are also observations of such processes in the Gulf of Mexico⁷; in Funka Bay in Hokkaido, Japan by H. Miyake, I. Tanaka and T. Murakami (personal communication); and in the Adriatic Sea in 1980, 1981 and 1983 by L. Zoccolotti and E. Salusti (personal communication). The profusion of such processes suggests that the aggregate effect contributes to the ventilation of the ocean.

It is probably not appreciated how widespread this process is: none of the above studies refers to the others. In all cases, references to theoretical and laboratory experiments investigating the dynamics of such flows are also lacking. The effect of the Earth's rotation on bottom-water formation was excluded in a civil-engineering study of lakes⁸, but included in another model applied to the oceans⁹. The rotation causes gravity currents to lean to the right in the Northern Hemisphere and to the left in the Southern Hemisphere. Measurements have verified formulae like the Chezy equation used by Lennon and colleagues¹. A similar formula with different theoretical and experimental bases, has been applied¹⁰ to the salt balance at Spencer Gulf. It predicts an excess of salt across the front in the mouth of the gulf that varies as a function of evaporation rate, and that agrees with the observed salinity. Such a calculation has also been verified for the front observed in Funka Bay.

The measurements by Lennon and colleagues have many implications beyond the local offshore oceanography they consider. This is true for the other studies as well. A combination of studies of local-gravity currents in various bays and continental-shelves, together with laboratory, numerical and theoretical studies, may even enable us to understand more clearly the situation in the Weddell Sea. □

1. Lennon, G.W. *et al.* *Nature* **327**, 695 - 697 (1987).
2. Foster, T.D. & Carmack, E.C. *Deep-Sea Res.* **23**, 301 - 317 (1976).
3. Tomczak, M. Jr *Continental Shelf Res.* **4**, 255 - 278 (1985).
4. Hendershott, M.C. & Rizoli, P.M. in *La Formation des Eaux océaniques profondes Colloques int. Cent. natn. Rech. scient.* no. 215, 135 - 138 (1974).
5. Zore, A. *Acta Adriatica* **10**, 1 - 94 (1963).
6. Fleux, M. *La Formation des Eaux océaniques profondes Colloques int. Cent. natn. Rech. scient.* no. 215, 165 - 174 (1974).
7. Nowlin, W.D. Jr & Parker, C.A. *J. phys. Oceanogr.* **4**, 467 - 486 (1974).
8. Brocard, D.N., Jirka, G.H. & Harleman, D.R.F. in *Ralph M. Parsons Laboratory for Water Resources and Hydrodynamics. tech. Rep. 223*, (Dept. of Civil Engineering, Massachusetts Institute of Technology, 1977).
9. Sugimoto, T. & Whitehead, J.A. *J. phys. Oceanogr.* **13**, 1819 - 1828 (1983).
10. Bye, J.A.T. & Whitehead, J.A. Jr *Estuarine and Coastal Marine Science* **3**, 477 - 481 (1975).

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Biological control

Pests, pathogens and weeds

R.K.S. Wood, M.J. Way and R.M. May

IN THE opening paper at a recent meeting*, J.K. Wagge and D.J. Greathead (Commonwealth Institute of Biological Control) told the roughly 500 attendees that today — a century after the first deliberate and successful introduction of a natural enemy to control a pest — control programmes face three new and interlinked challenges. First, there is growing public anxiety about the possibility that using pesticides, herbicides or genetically engineered organisms to control pests or weeds has unforeseen ill-effects. We are still learning how to combine good science with good political procedures to address such worries. Second, the underlying ecological theory is still in its infancy, and is suffering growing pains as it comes to grips with the realistic complications inherent in nonlinear dynamics and spatial heterogeneity. Third, the expanding diversity of kinds of control agents and of approaches compounds these first two problems.

Combining mathematical models with empirical studies in the field and laboratory, M.P. Hassell (Imperial College, London) and one of us (R.M.M.) showed how specific components of the behaviour of insect predators (foraging efficiency, sex ratio, response to environmental patchiness) and of the population biology of pathogens (transmissibility, virulence) influence biological control with such agents. We drew parallels between the dynamics of insect prey-predator and host-pathogen systems, emphasizing that predators or pathogens must be released at densities above some threshold value if they are to achieve local eradication of a target pest. We also presented field data and theory on differences between temperate-zone insect pests, which tend to have discrete, non-overlapping generations, and tropical pests, where many generations often overlap.

Turning to the control of specific insect pests, H.F. van Enden (University of Reading) observed that sentimental appeals to the "balance of nature" fail to recognize that, for example, the coevolution of aphids and their indigenous natural enemies is such that biological control to a level acceptable to growers of field crops will rarely occur in the absence of manipulative intervention. He went on to show that the ratio of natural enemies to aphids is more important than the absolute number of natural enemies present, and suggested ways of keeping this ratio high. P. Neuenschwander and H.R.

Herren (International Institute of Tropical Agriculture, Nigeria) described the depredations wrought by the cassava mealybug after its accidental introduction into Africa, and its so-far successful control by the specific parasitoid *Epidinocarsis lopezi*. This natural enemy was introduced from South America, the original home of the cassava mealybug; laboratory studies of the population dynamics of the parasitoid, together with exclusion experiments in the field, help explain why it is an effective control agent despite its low reproductive potential.

J.H. Lawton (University of York) reviewed the increasing problems caused by bracken as it spreads at 1–3 per cent per year in Britain, causing direct loss of grazing land, stock poisoning and shepherding difficulties in dense stands. He observed that biological control has very rarely been attempted against a native plant anywhere in the world, and went on to describe some insects found on bracken in South Africa that might control bracken if released from their natural enemies (as they would be in Britain). Lawton surveyed some of the broader problems associated with any such introduction, urging that there should be "a proper national assessment of the bracken problem, in which ecological and economic consequences of biological control can be weighed against the effects of continuing with present policies".

The less conspicuous fungal and bacterial pathogens of plants must struggle for existence with other microorganisms near or at the surfaces of their host plants, and pathogens in the soil face severe competition from the indigenous microflora. Intensive agriculture often creates conditions that tip the balance in favour of the pathogens, resulting in increasingly severe losses of crops in successive seasons. R.J. Cook (Washington State University) discussed ways in which the crop environment can be managed so as to minimize losses to pathogens, drawing examples from his successful control of root diseases of wheat in the Pacific north-west. Thus, resistance to foot rot, *Fusarium culmorum*, is increased by minimizing water stress in plants by judicious use of fertilizers in relation to available water. In contrast, seedling diseases caused by *Pythium* spp., which are more severe in wetter soils, are controlled by ensuring that upper layers of soil are sufficiently dry during the early growth of plants, and by managing crop residues so that they do not become food bases that would increase residual populations of the pathogens.

As mentioned by Cook, and reviewed more fully by J.W. Deacon (University of Edinburgh), harmful pathogens of plants can also be controlled by adding 'antagonists' of the pathogen to the soil. Such antagonistic microorganisms can be targeted against dormant propagules or against active populations of pathogens; in either case there are formidable difficulties in disseminating them through large masses of soil to act against pathogens that are usually distributed very non-uniformly. Application is less of a problem in diseases where antagonists can be placed close to where pathogens infect, as on seeds, in seed drills, on transplants or in holes in which seedlings are planted. Introduced organisms also can diminish disease by spreading extensively from sites of application to colonize root surfaces, and thus to exclude pathogens such as *Gaeumannomyces graminis* and other pathogens of wheat. J. Risbeth (University of Cambridge) surveyed the corresponding use of antagonistic microorganisms to control airborne pathogens of plants. He gave several specific examples where suspensions of commercially prepared microorganisms, including filamentous fungi, yeasts and bacteria, are applied to the leaf surfaces of crops or to wounds on trees. In particular, inoculating pine stumps with *Peniophora gigantea* has been successful in preventing colonization by the fungal pathogen *Heteroasidium annosum*.

Given the worries about pathogens that damage crops, why not use other pathogens to damage unwanted weeds? Such methods for controlling weeds have only recently been exploited on any scale (J.M. Cullen and S. Hasan, CSIRO Biological Control Unit, Montpellier). One notable success is the control of *Chondrilla juncea*, a pernicious weed introduced into Australia from Europe, by its European pathogen, the rust fungus *Puccinia chondiclinia*. Cullen and Hasan noted that, when pathogens are introduced to depress weed populations, great care must be taken in preliminary surveys to ensure the pathogen is specific to its target plant, and harmless to others. Despite these problems, and other practical difficulties associated with patents, storage, and activity and persistence in the field, there is expanding commercial interest in developing fungal pathogens (mycoherbicides) as alternatives to conventional herbicides.

Throughout the meeting, there was a tendency for those who deal with insect pests to give explicit consideration to the overall population dynamics of the interacting species, whereas those who deal with pathogens and plants focused more on detailed mechanisms in the interaction between individual organisms. This could be inherent in the different biology of the different situations, but we think the

* Biological Control of Pests, Pathogens and Weeds: Development and Prospects Royal Society discussion meeting, London 18–19 February 1987.

explanation is more likely to be that some researchers tend to have been educated in a population-oriented ecological tradition, whereas others have been educated in a physiological and mechanism-oriented tradition. If so, there could be room for some fruitful cross-fertilization.

A. Jutsum (ICI Plant Protection Division, Jealotts Hill) surveyed commercial applications of biological control. He observed that sales of biological control agents currently account for less than 1 per cent of the roughly \$16,000 million spent annually on products used in crop protection and public health. Jutsum emphasized the sharply targeted approach that industry brings to research and commercialization of control agents, and described successes and failures of products ranging from pheromones and specific control agents (which spare beneficial organisms) to mass-produced bacteria, predatory mites and other agents. He suggested that bacteria and fungi could have the greatest commercial potential, despite various problems of

registrability (particularly for genetically engineered agents), patentability, reliability and cost-effectiveness. Although biological control agents are not likely to replace chemicals in the foreseeable future, industry believes that biotechnology will increase the profitable use of these agents, and is working actively, in collaboration with academic researchers, to advance this technology. As this work progresses, we think there will be a growing need for studies of the ecology of microorganisms in controlled laboratory settings (as well as in the field), as part of the safety programmes that must accompany the release of genetically engineered organisms as control agents. Such systematic studies of laboratory 'microcosms' will require expenditures on a scale more customary for chemists and physicists than for ecologists. □

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Superconducting ceramics

Experiments show the way

J.-L. Tholence and B. Raveau

SINCE the discovery of high-temperature superconductivity in the two families (La_2CuO_4 and $\text{YBa}_2\text{Cu}_3\text{O}_7$) of oxide ceramics, a profusion of theories and experimental data have been published. More complex experiments are now being performed to elucidate the effect of different substitutions or of the oxygen vacancies (and whether they are ordered or disordered), and to test the new theories. A special day at a recent European conference* was devoted to high-temperature superconductivity. Both experimental and theoretical advances were reported, but as the forthcoming theoretical meeting at Berkeley is to be discussed soon in News and Views, here we will describe only the experiments.

In his introduction, J. Bednorz (IBM, Zurich) explained how his search, with K. Muller, for the high-temperature superconductivity arising from Jahn-Teller polarons was successful because of the presence of Cu^{3+} in ceramics synthesized by C. Michel and one of us (B.R.) at Caen University. The onset temperature, T_c , for superconductivity depends strongly on the $\text{Cu}^{3+}/\text{Cu}^{2+}$ ratio and the dimensionality of the Cu-O subsystem that links the conducting planes between the limiting formulae LaCuO_2 , $\text{La}_2\text{Ba}_2\text{Cu}_6\text{O}_{14}$ and $\text{YBa}_2\text{Cu}_3\text{O}_7$, with three-, two- and one-dimensional structures, respectively (D.M. de Leeuw, Philips, Eindhoven;

B.R.). High-resolution electron microscopy shows agreed inhomogeneity of the crystals (B.R.): most exhibit twin-domains, oriented domains and extended defects in phases both over and under stoichiometric in oxygen ($\text{YBa}_2\text{Cu}_3\text{O}_7$ -type and La_2CuO_4 -type).

Some of the properties of these ceramics (despite their high- T_c values and two-dimensional character) remain consistent with the familiar BCS theory based on electron-phonon interactions. Thermoelectric power (responsible for the action of thermocouples) has been observed above T_c and starts to decrease well above T_c (C. Uher, Univ. Stuttgart). Electron-tunnelling measurements (P.J.M. van Bentum, Univ. Nijmegen) indicate a superconducting bandgap (the difference in energy between the superconducting and normal states) $2\Delta \sim 3.5\text{--}4 kT_c$, where k is Boltzmann's constant. On the other hand, experiments by B. Batlogg and co-workers (AT&T Bell Labs; also *Phys. Rev. Lett.* **58**, 2333-2336; 1987) show the absence of isotope effect of oxygen (^{16}O , ^{18}O) on T_c , which appears to exclude the traditional electron-phonon mechanisms. (See the recent News and Views article by P.W. Anderson and E. Abrahams, *Nature* **327**, 363; 1987.) Low Debye temperatures (130-200 K) that characterize the phonon distribution, obtained from specific-heat measurements (P. Gutsmedl, W. Meissner Institut, Garching), imply low phonon frequencies

and also suggest that BCS-type coupling cannot explain the high T_c s.

The phase diagram of Y-Ba-Cu-O compounds is now better explored (Batlogg; J.D. Jorgensen, Argonne National Lab.). For $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ the structure, the oxygen positions and the transition from tetragonal to orthorhombic structure have been studied for $0 < \delta < 1$ (Jorgensen; M. Marezio, CNRS, Grenoble; also see Welch, D.O. *et al. Nature* **327**, 278; 1987). It is striking that T_c is maximum in $\text{La}_{1-x}\text{Sr}_x\text{CuO}_{4-\delta}$ with the composition at which the structure changes from tetragonal to orthorhombic (Bednorz; Jorgensen; Batlogg), but that T_c tends to zero for the oxygen composition corresponding to the same transition in $\text{YBa}_2\text{Cu}_3\text{O}_{7-\delta}$ (Jorgensen). It is now accepted that the oxygen-deficient phase $\text{La}_2\text{CuO}_{4-\delta}$ is semiconducting, and antiferromagnetic below the Néel temperature $T_N \sim 240$ K (H. Maletta, Univ. Jülich), and that T_N decreases with decreasing δ (D.C. Johnston, Exxon, Annandale). According to experiments at our laboratories, it appears that samples, heat-treated at 900°C in 500 bar of oxygen (and nearly stoichiometric in oxygen), are completely superconducting below $T_c = 33$ K; but the superconductivity is destroyed by only 1 per cent of impurity (strontium or yttrium) on the lanthanum sites. The question of whether the superconducting phase is still antiferromagnetic is important in distinguishing certain theories.

Rumours about higher transition temperatures in the yttrium-barium compounds have been substantiated by D. Djurek (Zagreb Univ.; reported by Bednorz), who found that the resistivity falls to zero at 220 K. C. W. Chu and co-workers (Houston Univ.; reported by Wenger) found that the resistivity decreases by steps at 240 K, 220 K and 190 K before becoming null below 90 K. L.E. Wenger (Wayne State Univ.) observed that the a.c. susceptibility of La-Ba-Cu-O samples is extremely sensitive to the a.c. field because of Josephson contacts between grains, and used this effect to perform reverse a.c. Josephson measurements to detect minority superconducting phases in Y-Ba-Cu-O mixtures that showed a drop in resistivity at 240 K, but he could not entirely exclude the possibility that he had observed some pseudopiezoelectric effect often observed in ceramics. Also superconductivity in fluorinated compounds ($\text{YBa}_2\text{CuF}_2\text{O}_7$) has been confirmed at 155 K (Ovskinsky, S.R. *et al. Phys. Rev. Lett.* **58**, 2579-2581; 1987).

Batlogg reported that the width of the resistive transition of polycrystalline samples increases with applied magnetic field. This is related directly to the anisotropy of the critical field, H_{c2} , that quenches superconductivity. This is ten

* 1987 meeting of the European Materials Research Society, 2-5 June 1987, Strasbourg.

times larger in the Cu-O plane than perpendicular to it in $\text{YBa}_2\text{Cu}_3\text{O}_7$ (H. Noel, Rennes Univ.). The gradient $-dH_c/dT$ increases with decreasing temperature, and also with increasing applied field from -2 T K^{-1} in low fields (5 T) to -3 T K^{-1} in 20 T (Noel; J.C. Ousset, CNRS, Pont à Mousson) and -4.2 T K^{-1} in 25 T (van Benthum). Superconductivity is often associated with a high degree of magnetic flux exclusion (the Meissner effect). In the new materials the extent of the Meissner effect can be very small (< 4 per cent) in completely superconducting samples with a narrow transition because of the strength of flux pinning when the sample was cooled in a magnetic field (P. Lejay).

Finally, the critical current I_c , at which superconductivity fails, is of great importance in applications of the new

materials. Several measurements of I_c in $\text{YBa}_2\text{Cu}_3\text{O}_7$ were reported. Batlogg expects the intrinsic bulk critical current to be much larger (10^6 A cm^{-2}) than the value he has recorded at 77 K, $1,100 \text{ A cm}^{-2}$. R.B. Laibowitz (IBM, Yorktown Heights) has found $I_c \sim 10^6 \text{ A cm}^{-2}$ in thin films at 77 K. Using magnetic hysteresis, values of 10^5 A cm^{-2} at 4.2 K and $1,100 \text{ A cm}^{-2}$ at 77 K have been obtained in polycrystalline samples (Lejay), and a value of 750 A cm^{-2} was obtained in a copper-coated wire (H. Yoshino, Toshiba). □

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Protein structure

One fold among many

Lindsay Sawyer

WHAT is the connection between insect camouflage, cow's milk and vitamin A? The answer is a binding protein, found in the first two and associated with the last. A paper published at the end of last year drew attention to the similarity between the crystal structures of the cow protein β -lactoglobulin (BLG) and plasma retinol-binding protein (RBP), where the striking 'cross-hatching' of the eight strands of anti-parallel β -sheet provides a protective cover for the labile and insoluble retinol (vitamin A) bound in the core of the protein¹. The particular polypeptide fold, which includes a helix near the carboxyl terminus, is a novel variation of all- β protein. Two independent determinations of the structure of insect-cyanin, or bilin-binding protein (BBP), from the butterfly *Pieris brassicae* by Huber and colleagues² and from the tobacco hornworm *Manduca sexta* by Holden *et al.*³, now expand and diversify the family.

The figure shows the backbones of the three structures in the same approximate orientation. The model of Huber and colleagues² is incomplete but the authors point out that there is some unexplained electron density in the core of the protein that is likely to be the pigment biliverdin, formed by the breakdown of haem. The structure of Holden *et al.* shows the biliverdin clearly but its horseshoe shape is something of a surprise because, by analogy with retinol in RBP, one might have expected the pocket to be long and thin. The function of the BBP is uncertain, although the colour is

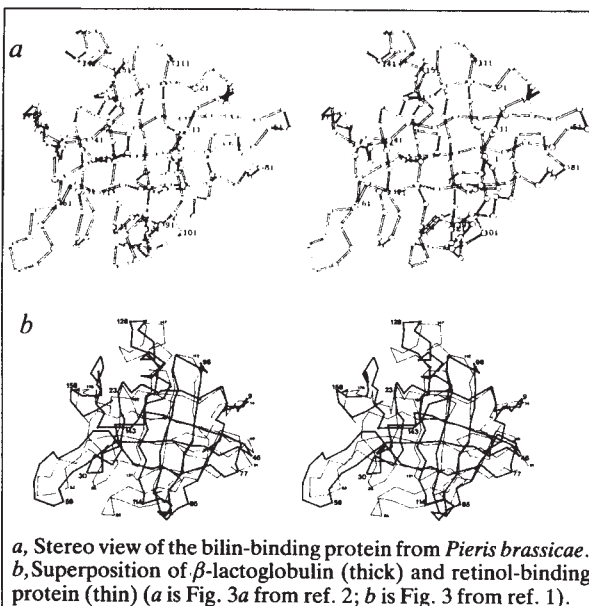
important for the camouflage of the insect. But as the ligand is not very soluble at neutral pH, this is also consistent with a role as a transporter.

Three diverse proteins sharing a common structure and possibly a similar function, that of transport, may seem little more than a coincidence. Sequence comparisons of the three proteins provide but

-U-X-X-Gly-X-Trp-Y- (U is basic, Y aromatic and X either), occurs near residue 20 and the sequence -Thr-Asp-Tyr-X-X-Y- appears around residue 110. Midway between these two peptides, a disulphide forms a bridge to the carboxyl terminus.

This pattern had already been noticed for BLG and RBP and was extended to include protein HC (or α -1-microglobulin)⁵ and α -2u-globulin, a urinary globulin. Two androgen-dependent secretory proteins from the rat epididymis also fit the pattern⁶ and apolipoprotein D, which comprises 5 per cent of the high-density lipoprotein involved in lipid transport in serum, has a sequence clearly related to BBP⁷. A protein found in the nasal mucus of the frog *Rana pipiens* also complies⁸. That this protein is somehow involved in transferring odorants to a receptor is a prediction based on its similarity to RBP and one which should hasten the completing of the sequence of bovine odorant-binding protein⁹. Finally, the lobster retinoid-binding protein crustacyanin also seems to be a member (J.B.C. Findlay, personal communication).

Ten distinct proteins so far show the features described and in the four for which DNA sequence information is available the pattern can be extended to include the disposition of intron/exon boundaries, thereby showing a strong evolutionary relationship¹⁰. The functions of these proteins are mostly unknown, but they all bind small, conjugated molecules that are either sparingly soluble, labile or both. The proteins are all of similar size and, it is reasonable to assume, the same shape. Further, the mammalian ones (at least) all seem to have an associated receptor. Thus, we might expect to find a protein formed into a calyx of eight strands of anti-parallel β -sheet wherever such extracellular transport occurs, whether it is for distribution or acquisition of metabolites, transduction of signals or removal of waste products. What is particularly exciting is that if this basic framework is especially suited to binding small, hydrophobic or fragile molecules, then it should be possible to engineer a transporter for a given molecule by starting from a conveniently cloned and expressed precursor such as β -lactoglobulin or apolipoprotein D. □



scant evidence for the similarity of their tertiary structures. In fact, although any pair has around 30 per cent of residues in common, there are less than a dozen residues common to all three. However, these common residues do form a pattern, although less well defined than the template for nucleotide binding⁴: in a protein of mass around 20,000, the sequence

1. Papiz, M.Z. *et al.* *Nature* **324**, 383-385 (1986).
2. Huber, R. *et al.* *J. molec. Biol.* **195**, 423-434 (1987).
3. Holden, H.M., Rypniewski, W.R., Law, J.H. & Rayment, I. *EMBO J.* **6**, 1565-1570 (1987).
4. Wierenga, R.K. *et al.* *J. molec. Biol.* **187**, 101-107 (1986).
5. Pervaiz, S. & Brew, K. *Science* **228**, 335-337 (1985).
6. Brooks, D.E. *et al.* *J. biol. Chem.* **261**, 4956-4961 (1986).
7. Drayna, D. *et al.* *J. biol. Chem.* **261**, 16535-16539 (1986).
8. Lee, K.-H. *et al.* *Science* **235**, 1053-1056 (1987).
9. Cavagioni, A. *et al.* *FEBS Lett.* **212**, 225-228 (1987).
10. Clark, A.J. & Ali, S. *J. molec. Biol.* (submitted).

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Particle physics

To observe a quark plasma

Helmut Satz

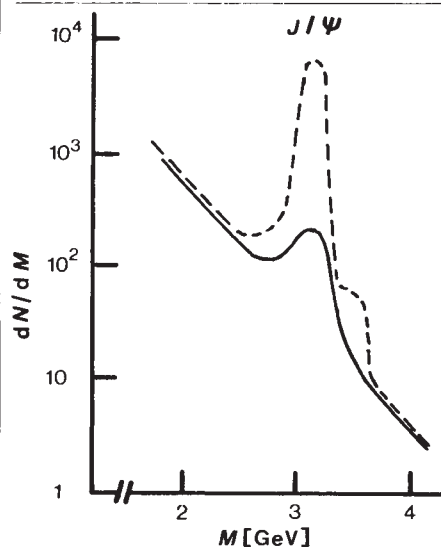
QUANTUM chromodynamics (QCD), the theory of strong interactions between elementary particles, predicts that at sufficiently high density, nuclear matter is transformed into a plasma of unconfined quarks. One of the great challenges to experimental physics today is to produce this novel state of matter in the laboratory and to study the transition from the plasma back to ordinary nuclear matter. High-energy collisions between nuclei should provide the necessary tool for this, as they are expected to produce bubbles of quark plasma. But how can one test that such collisions really lead to quark deconfinement? Is there an unambiguous, observable signal for quark plasma formation? Recent theoretical considerations^{1,2} indicate that there is, and the corresponding experiments are in progress at CERN in Geneva.

According to QCD, quarks in ordinary nuclear matter are always bound, or confined, in hadrons such as protons or pions. In sufficiently dense nuclear matter, however, the confining force is screened by the close proximity of many quarks. This screening, which is similar to the Debye screening found in ordinary ion-electron plasmas, prevents the binding of quarks into hadrons if the density is so high that the range of the interquark force is less than the radius of a hadron. If produced in a nuclear collision, a bubble of deconfined plasma will expand and eventually the density will drop low enough to allow quarks to bind again; at this point, the system hadronizes, reverting to ordinary nuclear matter. The expansion process is very rapid (lasting 10^{-21} s or less), so that the plasma cannot be measured directly; by the time of measurement, all quarks will have combined to form quark-antiquark pairs (mesons) or quark triplets (baryons).

So the final state always consists of hadrons. To find out whether these are produced by the usual nucleon-nucleon interactions, or if they are the final state of a quark plasma, we have to consider a rather special hadron, the J/ψ meson discovered 13 years ago by the groups of S. Ting and B. Richter. This is a bound state of the heavy 'charmed' quark c ($m_c \sim 1,500$ MeV) and its antiquark $\bar{c}$, in contrast to the usual mesons, which consist of light quarks q and antiquarks $\bar{q}$ ($m_q \sim 10$ MeV).

The production of a J/ψ in a nucleon-nucleon collision proceeds by two steps: a $c\bar{c}$ pair is created which then binds into a J/ψ . But if this process were to occur in a quark plasma, the binding of the c and $\bar{c}$ to

form a J/ψ would be impossible, as the binding force would be screened, and they would simply fly apart. At the hadronization point, when the quarks start to combine into hadrons, J/ψ formation is still almost impossible. Further production of c or $\bar{c}$ quarks is highly suppressed in the thermal medium because of their large mass, and the charmed quarks and antiquarks formed initially will have separated and will therefore combine with light



Production rate dN/dM for lepton pairs (e^+e^- or $\mu^+\mu^-$) of mass M , as observed in proton-proton collisions (broken curve). The solid curve gives the corresponding distribution expected for lepton-pair production in nuclear collisions leading to significant formation of quark plasma. The latter distribution is normalized so that the non-resonant background coincides with that of the proton-proton case.

quarks and antiquarks to form $c\bar{q}$ or $\bar{c}q$ mesons, not the J/ψ . So a strong suppression of J/ψ production in nuclear collisions would be a signature of quark plasma formation.

For this signature to be unambiguous we need to be sure that other mechanisms do not lead to the same effect. For example, could J/ψ s be absorbed by nuclei, mimicking the suppression? Measurements of the interaction of J/ψ s with nuclei³ indicate that this does not happen; neither do we know of any other suppression mechanism. So the strength of J/ψ production seems to be a clear indicator of the presence or absence of quark deconfinement.

The J/ψ , moreover, is easily detected, as it decays into lepton pairs (e^+e^- or $\mu^+\mu^-$) showing a strong, pronounced mass-spectral peak. Suppression through plasma formation does not imply that the J/ψ peak will not be observed (see the

figure); peripheral non-plasma events will always generate some J/ψ particles. But by using heavier nuclei to give more plasma or selecting events with higher multiplicity of hadron production (associated with higher plasma density), the suppression should be more effective. The variation of experimental parameters will thus allow a detailed test of quark deconfinement.

If nuclear collisions do produce quark plasmas, how can we identify the thermal conditions there? Again, the study of lepton-pair emission is a very suitable approach⁴ and, for this reason, has been investigated in considerable detail^{5,6}. The quarks in the early plasma stage emit virtual photons (non-classical fluctuations in the electromagnetic field), that subsequently decay into lepton pairs. As these interact with quarks only by comparatively feeble electromagnetic forces, they generally escape from the plasma without any further interaction. Hence such thermal dileptons are an ideal thermometer⁷ for measuring the initial temperature of the strongly interacting matter formed in nuclear collisions.

Lepton pairs can also be produced by non-thermal processes similar to the $c\bar{c}$ formation by a hard scattering of quarks in the incident nuclei. To use the dilepton spectrum as a measure of plasma temperature, we must be sure that the leptons are of thermal origin. Some indication is given by the functional form of the spectrum, but a more clear-cut test was proposed recently⁸. Non-thermal processes generally result in lepton pairs whose momenta are aligned with the incident-beam direction. Thermalization destroys the memory of this direction and hence leads to isotropic emission of lepton pairs, so that the angular distribution would be a test of their thermal origin. The lepton-pair masses for thermal emission generally fall in the intermediate range below 2–2.5 GeV, so it is not expected that thermal production will be significant in the energy range where J/ψ suppression occurs. On the other hand, the lepton-pair spectrum may well provide decisive information about different aspects of quark-plasma formation over the entire mass range.

The first experimental search for quark plasmas in nuclear collisions was performed last autumn at CERN. Two large collaborations there (NA34 and NA38) have facilities to measure lepton pairs and could detect J/ψ suppression as well as

1. Matsui, T. & Satz, H. *Phys. Lett.* **178**, 416–422 (1986).
2. Karsch, F. & Petronzio, R. *CERN report TH.4699/87*, 1–10 (April 1987).
3. Branson, J.G. et al. *Phys. Rev. Lett.* **38**, 1334–1337 (1987).
4. Feinberg, E.L. *Nuovo Cim.* **A35**, 391–412 (1976).
5. Kajantie, K., Kapusta, J., McLerran, L. & Mekjian, A. *Phys. Rev. D* **34**, 2746–2754 (1986).
6. Gorenstein, M.I. & Pavlenko, O.P. *Acad. Sci. Ukrainian SSR Report ITP-87-3E*, 1–9 (January 1987).
7. Kajantie, K. & Miettinen, H.I. *Z. Phys.* **C9**, 341–346 (1981).
8. Hoyer, P. *Phys. Lett.* **B187**, 162–164 (1987).

measure the plasma temperature. These first experiments, performed with an oxygen (^{16}O) beam of 200 GeV per nucleon incident on heavy targets (lead or uranium), are being analysed now. Later this year further data will be obtained using a sulphur (^{32}S) beam of the same energy. It may well be that both oxygen and sulphur are too light to form quark plasmas, but by considering only high-multiplicity events,

corresponding to a higher initial density, some first indications might be obtained. In any case, both at CERN and at Brookhaven National Laboratory in the United States, experiments with beams of much heavier nuclei are being planned. □

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Ageing

X-chromosome reactivation

Robin Holliday

It has often been suggested that ageing results from accumulated defects in certain macromolecules or cellular structures. Because the survival of higher organisms depends on the normal functions of differentiated cells, an important possibility is that ageing impairs or disrupts the necessary controls of gene expression. On page 725 of this issue¹, Wareham *et al.* describe a simple but powerful technique to examine the stability of X-chromosome inactivation during the ageing of mice. Their results show that a silent gene on the inactive X chromosome is reactivated with increasing frequency during ageing. This can be regarded as an epigenetic error at the DNA level, presumably because of the failure of normal protein-DNA interactions.

During the early development of female eutherian mammals, there is random inactivation of one of the two X chromosomes in each cell. This inactivation is stably inherited through mitotic division, so that all descendants have the same

active X chromosome (either paternal or maternal) and the same inactive one, with the result that each adult female is a mosaic with regard to X-chromosome activity. The inactivation process is an excellent example of a stable epigenetic switch, as the two chromosomes have essentially the same genetic information and reside in a common nucleoplasm and cytoplasm.

It has been known for a long time that autosomal DNA inserted into an X chromosome is also subject to inactivation. In the mouse insertional translocation described by Cattana², for example, the autosomal DNA contains the wild-type tyrosinase gene, which produces an albino phenotype when inactivated. Cattana² made the remarkable observation that as animals aged the tyrosinase gene is often reactivated, so that animals become more pigmented with age (the reverse of one well-known effect of ageing on hair pigmentation). This observation has now been extended by Wareham

et al., who demonstrate age-related reactivation of a normal X-linked gene.

The new study depends on the use of Searle's X-autosome translocation, which abolishes mosaicism of female animals. When the X chromosome with the translocation is inactivated in an embryo cell, an unbalanced, lethal genome is produced. Thus, adults consist only of cells in which the normal X chromosome is inactive. The experiment of Wareham *et al.* used the X-linked *spf* (sparse fur) mutation in the structural gene encoding the enzyme ornithine carbamoyl transferase (OCT). The mutation produces an abnormal form of the enzyme which is inactive at pH 7.0. Histochemical staining of enzyme activity in liver cells at this pH, using heterozygous animals (*spf*+) with random X-inactivation, as expected, yields preparations that are mosaic for enzyme activity. Mice that are also heterozygous for Searle's translocation, with the *spf* mutation on the X-autosome-translocated chromosome, yield non-mosaic animals with negative staining of liver tissue.

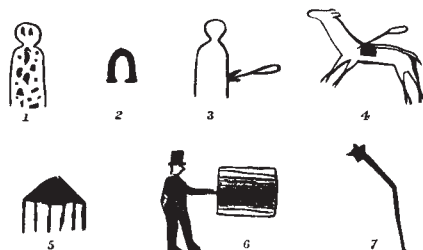
When Wareham *et al.* examined animals of different ages (8–10-week-old young adults; 6–9-month-old mature adults; and 14–17-month-old adults), they saw a minute proportion of stained cells in the young animals, a fivefold increase in mature adults and a further tenfold increase in old adults. A study of stained patch sizes shows that this result is not caused by selection of cells with the wild-type *oct* gene. In an important control, male *spf*/Y animals were found to be negative for stained cells, showing that that mutation to the wild type is not responsible for the stained phenotype. The experiments of Wareham *et al.* therefore provide the first clear-cut evidence for reactivation of an inactive X-linked gene *in vivo*, and their results indicate that reactivation is not linear with time, but accelerates as adult animals age.

It has often been proposed that ageing is caused by the random accumulation of errors, defects or damage in macromolecules. Chromosome abnormalities³ and somatic mutations⁴ increase during human ageing, and epigenetic control of gene expression is also vital to maintain the phenotype of specialized cells — the loss of such controls could contribute to the ageing process. There is convincing evidence that X-chromosome inactivation is related to differential methylation of cytosine in the DNA of the two X chromosomes⁵, and it is also known that the pattern of methylated sites is inherited through the action of a maintenance trans-methylase. The demethylating agent 5-azacytidine can reactivate genes on the inactive X chromosome in somatic cell hybrids, as well as other silent genes⁶. Methylation of intracisternal A particle DNA in mice shows that it decreases about fivefold between 6 months and 26

100 years ago

AMERICAN INDIAN PICTOGRAPHS

ONE of the most striking features of the present work is the account it gives of the newly-discovered custom of the Dakota Indians to keep national calendars. During the dreary periods of their six winter months, certain elders of the tribe amused themselves with talking over the events of the past year, and Lone-Dog discussed with them which of those events should be selected as the representative of that period. Suppose it was an outbreak of the small-pox: then Lone-Dog drew the outline of Fig. 1 on the part of his buffalo robe, and dabbed it with red spots. Then that year became ever after known as the small-pox year. Lone-Dog's calendar is particularly graphic. It covers the entire period from 1800 to 1871. The first entry is made in the middle of the robe, and the others are arranged in an oblong spiral. They are drawn in black and red, the latter usually representing blood, of which plenty seems to be spilt in murder or in hunting. Thus Fig. 3 is a case of murder; Fig. 4 is a year in which a vast number of elk were killed. Fig. 6 tells us that striped Spanish blankets were first introduced in that year by a trader. Fig. 7 is the year of a great aërolite.



When pictorial nick-names are given to each successive year in council the process must be very like that of giving nick-names to new boys at school. I have a vivid recollection of two old Etonians describing how it was done in their time. The new boy was well looked over and watched. Then, as the fancy took them, first one lad and then another addressed him with tentative nick-names. At length one stuck and the new boy was fitted with a generally-accepted name, by which he was afterwards known during the whole time he was at school.

This volume is an excellent example of the growing variety and wealth of material now available to enquirers into the origin of language.

FRANCIS GALTON

From *Nature* 36, 155–157; 16 June 1887.

months of age⁷. The level of cytosine methylation also steadily declines during the limited lifespan of human fibroblasts *in vitro*, which may be related to the ageing of these cells⁸⁻¹⁰. Thus, the results of Wareham *et al.* could be explained by the loss of methylation at critical sites, perhaps from DNA damage or reduced maintenance transmethylation activity.

Whatever their molecular basis, the observations clearly show that there is not a gradual loss of control in all cells (as this would lead to intermediate stained phenotypes), but rather to random events that produce a fully active *odc* gene. In other words, there is an all-or-none effect on the transcription of this particular X-linked gene. An obvious next step is to examine

two or more linked genes on the X chromosome to see whether reactivation occurs at individual sites during ageing, or in whole regions of DNA. □

1. Wareham, K.A., Lyon, M.F., Glenister, P.H. & Williams, E.D. *Nature* **327**, 725-727 (1987).
2. Cattanaach, B.M. *Genet. Res.* **23**, 291-306 (1974).
3. Jacobs, P.A., Brunton, M., Court Brown, W.M. Doll, R. & Godstein, H. *Nature* **197**, 1080-1081 (1963).
4. Morley, A.A., Cox, S. & Holliday, R. *Mech. Age. Dev.* **19**, 21-26 (1982).
5. Monk, M. *BioEssays* **4**, 204-208 (1986).
6. Jones, P.A. *Cell* **40**, 485-486 (1986).
7. Mays Hoopes, L.L., Brown, A. & Huang, R.C.C. *Molec. cell. Biol.* **3**, 1371-1380 (1983).
8. Wilson, V.L. & Jones, P.A. *Science* **220**, 1055-1057 (1983).
9. Holliday, R. *Expl Cell Res.* **166**, 543-552 (1986).
10. Fairweather, D.S., Fox, M. & Margison, G.P. *Expl Cell Res.* **168**, 153-159 (1987).

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Neural networks

Understanding physicists' brains

Gérard Toulouse

THE goals of neural network theory are to understand how brains work and to design devices that emulate some of their computing attributes. The suggestion that physicists might usefully contribute to this effort is not new. A recent debate^{1,2} provides us with an opportunity to draw some perspectives on the value of their contribution.

John von Neumann³ 40 years ago predicted that theories of computation would come to resemble thermodynamics in many respects. Shortly thereafter, the analogy between interacting neurons and coupled magnetic dipoles was elaborated⁴. In its simplest version, a magnetic moment is a two-state element (up or down). Similarly, a formal neuron can be thought of as an all-or-none device (firing or quiescent). In a neural assembly, each neuron adjusts its state according to the summed inputs it receives from other neurons, much as a magnetic atom aligns its moment along the resultant field created by its neighbours in a magnetic material.

The branch of physics concerned with the collective properties of large assemblies of particles is statistical mechanics, from which standpoint several assaults on neural-network modelling have been made. The latest invasion was triggered by John Hopfield⁵, a physicist turned biologist, and since then the main participants have been theoretical physicists with expertise in the statistical mechanics of disordered systems, such as spin glasses, materials with randomly interacting atomic magnetic dipoles.

The emphasis has been on the study of networks that perform pattern-recognition tasks — memory nets (see the recent News and Views articles of Anderson⁶ and of Sutherland⁷). The problem is to choose

the interneural couplings such that the persistent states of activity of the network, its attractors, correspond to desired (to be stored) activity patterns. Then, starting from an initial configuration of activity induced by external stimulation, the network state will evolve according to its internal dynamics towards a final state, by definition an attractor. This is the simplest example of what is called 'computing with attractors'. If the storage has been properly constructed, the network functions as a content-addressable memory that can recognize incomplete stimuli and restore the missing information.

To tackle these problems, statistical mechanics has had to evolve. Traditionally, statistical mechanics works the other way around. The couplings are given parameters, and the problem consists in finding the stable states. This is not easy, except for simple regular structures. But in the study of spin glasses, where the couplings are random variables, powerful new methods are used, and enough flexibility is gained to attack the memory problem. What has statistical mechanics brought so far to neural-network theory? The answer is on three levels.

First, there has been an enlargement of perspectives (large-size effects, the inclusion of feedback architecture and asynchronous dynamics). The size of a network is the number of its constituent neurons. A century of statistical mechanics has shown the rewards of considering the apparently hopeless limit of a very large number of elements (in this limit only are phase transitions properly described). Feedback architecture and asynchronous updating (in contrast with the feedforward synchronous restrictions of more primitive architectures) are important features naturally accommodated in the new framework.

Second, the growing repertoire of exactly soluble models brings surprises and raises new questions. Science has been called the art of the soluble; but statistical mechanics has often illustrated the virtues of the exactly soluble that reveals unexpected hard facts. The surprises here have appeared in the richness of the phase diagrams (obtained for different learning rules, the algorithms that program the interconnections) and in the diversity of working regimes of a memory net^{8,9}. Among the issues now being investigated are: optimal versus ultimate storage capacities; one-shot versus slow learning; strict-stability versus error-tolerant storage. These are technical terms, but the point is that these conceptual distinctions have now acquired precise and operational meaning, through the study of different models.

Third, the standards of theoretical quality have risen. The comparison between two rival learning algorithms is now studied quantitatively, and the pros and cons fully assessed. Poorly substantiated claims and *ad hoc* theorising meet more educated criticism. The progress is not only in the answers, but also in the clarification of the questions.

In return, neural-network theory has opened new vistas to statistical mechanics. Early hypotheses of the Hopfield formulation included symmetrical couplings (to define an energy function, the cornerstone of the canonical formalism of statistical mechanics) and complete connectivity (to use the simplifications of mean field theory). Biologists did not like these assumptions. Indeed, not only has it been possible to relax those restrictions, but unexpectedly, it is often easier to do away with them and to study models with asymmetrical and diluted interactions.

The European Economic Community, prompted in part by the boom in neurocomputers in the United States and the Japanese 'human frontiers' programme, has launched a new initiative called BRAIN, basic research in adaptive intelligence and neurocomputing. One of its intentions is to promote cooperation among biologists, computer scientists and physicists studying networks of real and formal neurons. □

1. Hopfield, J.J. & Tank, D.W. *Science* **233**, 625-633 (1986).
2. Hopfield, J.J. & Tank, D.W. *Science* **235**, 1228-1229 (1987).
3. von Neumann, J. in *Collected Works*, Vol. 5, 304 (Pergamon, New York, 1963).
4. Cragg, B.G. & Temperley, H.H.V. *Electroencephalogr clin Neurophys* **6**, 85-92 (1954).
5. Hopfield, J.J. *Proc. natn. Acad. Sci. U.S.A.* **79**, 2554-2558 (1982).
6. Anderson, J.A. *Nature* **322**, 406-407 (1986).
7. Sutherland, S. *Nature* **323**, 486 (1986).
8. Amit, D.J. in *Heidelberg Colloquium on Glassy Dynamics and Optimization* (Springer, Berlin, 1987).
9. Sompolinsky, H. & Kanter, I. *Phys. Rev. A* **35**, 380-392 (1987).

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How many nuclear reactor accidents?

SIR—My complaint¹ about Islam and Lindgren's procedure² for estimating the probability of a future nuclear accident was that they had ignored the distinction between probability and likelihood, thereby committing a logical error which had led them to estimate the probability with unjustified precision.

Subsequent correspondents have not taken this point. Schwartz³ integrated the normalized likelihood function in order to obtain what he called a "confidence limit", implicitly adopting a bayesian approach with a uniform prior distribution for r , the rate parameter of the Poisson process.

Fröhner⁴ suggested that I had disregarded "the correct prior" when in fact my point was that no prior is correct, and that it is wrong to apply bayesian methods to this problem.

Chow and Oliver⁵ stated that I had implicitly used a uniform prior distribution for $P = 1 - e^{-rT}$, the probability of one or more accidents in T reactor-years, "which is equally dubious" (that is, as dubious as using a uniform prior for r). But I did no such thing, and once again there has been a failure to distinguish between likelihood and probability. (They went on to consider further information which was not in the specification of the original problem and on which I will not comment here.)

I must emphasize that a graph of a log-likelihood or 'support' function such as I presented is not a probability distribution, cannot be integrated, and does not depend on any bayesian prior distribution. Likelihood analysis has a long history⁶, stretching back well before the recent resurgence of bayesianism which is sweeping through physics. The reactor example is an excellent one for clarifying the difference between the two types of analysis.

Fröhner mistook what the argument is about. It is not about how to choose an "uninformative" prior (by using "modern techniques for the assignment of prior probabilities which have a solid foundation in either group theory or information theory"), but about whether it is justifiable to use a probability distribution to represent ignorance at all. Nothing in group theory or information theory addresses this vital point.

R. A. Fisher⁷ stated in this connection that "no experimenter would feel he had a warrant for arguing as if he knew that of which in fact he was ignorant." Bayesian risk analysis is contaminated by this logical fallacy. Physicists have been seduced by its extreme elegance and simplicity, those quite proper arbiters of physical theories; but theories of inductive inference are not to be selected on such

grounds.

Bayesian risk analysis is but Laplace's Rule of Succession cloaked with the modern trappings of group theory and information theory, but it is no better founded than it was in 1854 when George Boole⁸ observed that the appearance of the arbitrary constants which are characteristic of bayesian theory "seems to imply, that definite solution is impossible, and to mark the point where inquiry ought to stop."

Surely the last thing to which we should apply doubtful logic is the recurrence risk of nuclear accidents.

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1. Edwards, A. W. F. *Nature* **324**, 417–418 (1986).
2. Islam, S. & Lindgren, K. *Nature* **322**, 691–692 (1986).
3. Schwartz, J. *Nature* **324**, 622 (1986).
4. Fröhner, F. H. *Nature* **326**, 834 (1987).
5. Chow, T. C. & Oliver, R. M. *Nature* **327**, 20–21 (1987).
6. Edwards, A. W. F. *Likelihood* (Cambridge University Press, 1984).
7. Fisher, R. A. *Statistical Methods and Scientific Inference* (Oliver & Boyd, Edinburgh, 1956).
8. Boole, G. *An Investigation of the Laws of Thought* (Walton & Maberly, London, 1854).

Schrödinger and *What is Life?*

SIR—In his commentary (*Nature* **326**, 555–558; 1987) on Schrödinger's book, *What is Life?* Max Perutz concluded that "a close study of the book and of the related literature has shown me that what was true in his book was not original, and most of what was original was known not to be true even when it was written". Furthermore, "In retrospect the chief merit of *What is Life?* is its popularization of the Timofeeff, Zimmer and Delbrück paper that would otherwise have remained unknown outside the areas of geneticists and radiation biologists". As someone who spent some years around 1950 working with both Schrödinger and Delbrück, I would like to comment briefly on these strong criticisms, which are quite wrong in my view.

In *What is Life?*, Schrödinger managed in a slim volume to resurrect the ideas of Timofeeff, Zimmer and Delbrück, indicating that genes must be considered as large molecules, to liken these molecules to an aperiodic crystal, to introduce the idea of a genetic code, and to discuss some of the thermodynamic problems posed by these ideas with regard to the stability of living systems. Not all the ideas may have been original, or all strictly correct (after all Schrödinger was a physicist discussing biological problems) but what the book did was to formulate these ideas in such a way that they gave a sense of excitement about the future perspectives of biology to established biologists as well as to non-

biologists, students and laymen. This is the chief merit of the book and the reason why it has become one of the few classic volumes in popular science.

This ability to conjure up vivid imagery, and Perutz's failure to appreciate its merit, is reflected in that part of his commentary which deals with Schrödinger's introduction of the "famous hypothesis that the gene is a linear one-dimensional crystal, but lacking a periodic repeat: an aperiodic crystal". Perutz goes on to wonder why Schrödinger did not adhere to Delbrück's much better formulation of "a polymeric entity that arises by the repetition of identical atomic structures". The reason seems clear. Schrödinger's presentation was aimed at a wide audience and created a picture that even today manages to stimulate new readers. Delbrück was writing a scientific paper, and although his version was possibly more correct it was also eminently forgettable.

Where Schrödinger admittedly did go wrong was in the arguments that led him to postulate that "Living matter, while not eluding the laws of physics as established to date, is likely to involve other laws of physics hitherto unknown which, however, once they have been revealed, will form as integral a part of this science as the former". It is important, however, to realize that this conclusion was not based, as implied by Perutz, on the supposedly unpredictably erratic behaviour of a small molecule such as a gene, but on the problem of biological order, by which in Schrödinger's words, "a single group of atoms existing only in one copy produces orderly events, marvellously tuned in with each other and with the environment according to most subtle laws". Schrödinger's contribution to the debate on order in biological systems has been discussed by Jacob (*The Logic of Living Systems*, Ch. 5, Allen Lane, 1974), and more recently in a perceptive review by Yoxen (*Hist. Sci.* **17**, 17–52; 1979).

The idea that new physical laws might be needed in order to explain the behaviour of living systems was not unique to Schrödinger and persisted for a long time after the publication of *What is Life?* In 1958 I was working at the Massachusetts Institute of Technology, and Bohr, who was then 73, came and gave a special series of lectures ranging over physics and philosophy. As part of the festivities, Delbrück (who was viewed with varying degrees of adulation, affection and awe by scores of scientists who had been at the California Institute of Technology) was asked to deliver a lecture dealing with Bohr's influence on biology. In this lecture Delbrück (who was a disciple of Bohr) first reviewed the then current research of Benzer on the fine-structure analysis of the *rII* genes of phage T4, and went on to argue that as the analysis de-

veloped even further any satisfactory genetic theory would have to invoke a new type of biological uncertainty principle. In the discussion which followed Bohr stood up, and much in the manner used in seminars so often by Delbrück, stated simply "I don't believe a word of it".

NEVILLE SYMONDS

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Influenza viruses and comets

SIR—Henderson *et al.*¹ congratulate themselves in the belief that they have demolished our hypothesis that influenza viruses may be connected with comets². Their argument, ostensibly based on phylogeny, is contingent on constraints that they themselves have imposed. Consequently, their conclusions have no relevance to our original cometary model. The assumption that "the simplest comet model is that different isolates of H1 influenza are carried on different comets, having developed independently . . ." is not one to which we ourselves would subscribe, nor are any of the subsidiary postulates they consider.

Contrary to the statement that there is a lack of a "slow-mixing compartment in the atmosphere", we have repeatedly shown that viral-sized particles introduced into the upper atmosphere from outside have a residence time of a decade or more. Such particles are pulled down seasonally, following closely the patterns of atmospheric circulation on a global scale. Seasonal influenza peaks separated by six months in opposite hemispheres are fully consistent with this picture.

There is no requirement in our model for viral additions from comets to take place any more frequently than about once a decade, corresponding to the observed major genetic shifts in the influenza virus. The more frequently occurring changes (designated genetic drift), which are essentially what Henderson *et al.* have mapped, are in our view no more than a sorting out of an initial viral mix according to developing immunities in host populations.

With each cometary inoculation a wide range of closely related viral sequences will be introduced into the upper atmosphere to be pulled down subsequently to ground level. The immunity systems of host species are thus confronted on a seasonal basis with a range of viral genotypes, but only one or two would be permitted to 'take' in any particular season. Such a selective response of the lymphoid system, which is the basis of the immune mechanism, would appear to be well documented. As immunity evolves, influenza sub-types circulating earlier will become replaced in subsequent seasons by

sub-types that would appear to be slightly altered. The 'evolutionary' changes charted by Henderson *et al.* are therefore irrelevant to the evolution of the virus but are connected with a progressive development of immune responses in host species.

Next, it is worth noting that the number of independent comets responsible for carrying the influenza virus need not be as large as it has been assumed. We have recently speculated that emissions from different parts of the same comet occurring in different transits may contribute to inoculations that lead to the observed genetic shifts³.

The strongest evidence against a purely Earth-bound theory of influenza is to be found in data that in our view goes decisively against a simple person-to-person transmission hypothesis. All the available epidemiological evidence is consistent with a model where the causative agent (or a trigger for it) is airborne and is brought down seasonally from a reservoir established in the stratosphere⁴.

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1. Henderson, I.M., Penny, D. & Hendy, M.D. *Nature* **326**, 22 (1987).
2. Hoyle, F. & Wickramasinghe, C. *Diseases from Space* (Dent, London, 1979).
3. Hoyle, F. *J. R. Soc. Med.* **79**, 691 (1986).
4. Hoyle, F., Wickramasinghe, C. & Watkins, J. *Viruses from Space* (University College Cardiff Press, 1986).

Values in Taylor's law of spatial distribution

SIR—Downing¹ has recently recommended that the values of b in Taylor's^{2,3} empirical law $s^2 = am^b$, describing the relationship between the sample variance s^2 and the sample mean m of the numbers of animals or plants of a species, obtained from groups of replicate samples, should not be used in comparing different mechanisms underlying the spatial distribution of natural populations, owing to statistical bias. However, if different probability models lead to different ranges of values of the parameter β in a relationship of the form $\sigma^2 = a\mu^\beta$, where μ and σ^2 denote the true (population) mean and variance of the counts within a group rather than their sample estimates, it may nonetheless be pertinent to use β as a basis for distinguishing between models. Downing uses the slope of a linear regression of $\log s^2$ against $\log m$ as his estimator b of β , and this may well be a biased procedure. However, the estimator b is not the only estimator of β to be used in the literature⁴, nor does bias in b preclude the existence of more reliable estimators of β . Indeed, simply applying bias-correcting techniques to b , such as the jack-knife or bootstrap, might be expected to yield better results. Furthermore, the bias of potential estimators of β in

small studies could be directly investigated, by examining their performance when used with randomly chosen subsets of the large datasets for *Papillia japonicum* and *Pyrausta nubilalis* discussed by Downing¹.

Choosing the correct model underlying spatial distribution is a difficult problem; distinguishing between parameters and their estimates, as elsewhere⁵ in ecology, at least makes the problem clearer.

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1. Downing, J.A. *Nature* **323**, 255–257 (1986).
2. Bliss, C.I. *J. Econ. Ent.* **34**, 221–231 (1941).
3. Taylor, L.R. in *Statistical Ecology* Vol. 1 (eds Patil, G.P., Pielou, E.C. & Waters, W.E.) 352–372 (Pennsylvania University Press, 1969).
4. Taylor, L.R., Woiwod, I.P. & Perry, J.N. *J. Anim. Ecol.* **47**, 383–406 (1978).
5. Schatzmann, E., Gerrard, R. & Barbour, A.D. *IMA J. Math. appl. Med. Biol.* **3**, 99–113 (1986).

Against 'pragmatype'

SIR—The problem experienced by R. H. L. Disney (*Nature* **326**, 251; 1987) is real and encountered by many fellow researchers. A type specimen has to ensure the continued and correct recognition of members of sibling species groups too.

The problem is, what procedure should be followed if the holotype of the first described species of the complex could be assigned with certainty to any of the newly discovered siblings. It is true that a new suitably preserved specimen has to be selected, but do we need a new type of category for this? *The International Code of Zoological Nomenclature* (Vol. 163, Recommendation 75E; 1985) suggests the nomination of neotypes in the case of *nomina dubia* even if type specimens of the nominal species exist.

But do we need to create a new type category, such as Disney's suggested 'pragmatype', to mark such specimens? After all, the holotype of *Megaselia pulicaria* (Fallén) on a pin was in its time as much a pragmatic designation as the same on a slide today. Moreover, the new slide preparation would become an impediment to sensible decisions itself as soon as somebody splits the same taxon further, based on for example electrophoretic characters. A new type would have to be designated again, and if we are consistent yet again under a new type category.

The code covers exactly this and similar situations. It is recommended that neotypes should be designated for *nomina dubia* if, despite the existence of a holotype or a lectotype, it is not possible to resolve a complex zoological problem.

I think our problem and the aims of zoological nomenclature are best served by following the offered solution.

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Big physics in Europe

H.B.G. Casimir

History of CERN, Vol. I. By A. Hermann, J. Krige, U. Mersits and D. Pestre.
North-Holland: 1987. Pp.600. DG250.

FROM its beginnings, CERN at Geneva has been the pride of the European physics community. Its accelerators are the most powerful and refined in the world, and the instrumentation required for observing and measuring the properties of the particles produced by them is as good as that to be found anywhere. Excellent scientific work has been and is being done by its truly international staff, as well as by visiting teams from many countries. This was underlined by the recent award of Nobel prizes to Carlo Rubbia and Simon van der Meer. No wonder that CERN is proclaimed an outstanding example — and, physicists are inclined to add, the only real example — of a highly successful international undertaking.

How did this establishment come into being? As early as 1950 a small group of imaginative physicists, reacting to a fervent plea for European collaboration voiced by the American physicist Isidor Rabi at a UNESCO meeting, saw both the necessity and the feasibility of a European laboratory for high-energy physics. Soon they were joined by colleagues from several countries, and in those days governments were keen on international collaboration and were far-sighted enough to accept almost unquestioningly the plans proposed by the scientists. That, in a nutshell, is the received history. Things were not as simple as that, however, and part of the received history is no more than myth.

Several of the pioneers have written about the origins of CERN. Their reminiscences are valuable but have their limitations. Human memory is unreliable and no one can entirely escape a tendency to reconstruct the past to accord with one's present views. A true history remained to be written. A project launched in 1972 was abandoned in 1977, but soon afterwards a new advisory committee was set up, a feasibility study was carried out by Armin Hermann, the highly experienced professor of the history of science and technology at Stuttgart, his plans were accepted, financial support was found — the writers were not to be on the CERN payroll — and on 1 October 1982 a newly established study team started work. A year later it consisted of the authors mentioned above.

The complete study will cover the history of CERN up to the mid-1960s. This first volume deals with the years 1949–1954, that is with the birth and the official establishment of the centre at

Geneva. The team tackled the subject as true historians should. They did make use of interviews, personal recollections and so on, but first of all they carefully studied a vast number of documents and were able to reconstruct what actually happened almost month by month.

In the aftermath of the Second World War there was among European physicists an almost universal willingness to promote international collaboration, but

the attitudes of scientists and governments and the reasons for finally approving the plans differed from country to country. Individual chapters deal with the situation in Britain, Germany, France and Italy.

A curious problem arose in connection with the name of the organization. The acronym CERN was accepted by all concerned, but opinions differed about what it stood for. Some interpreted it as "Centre Européen pour la Recherche Nucléaire" but others as "Conseil Européen . . ." (Auger facetiously drew up a long list of other possibilities, including "Casino", "Concubinage" and "Cirque"). The highest authority in the organization, the CERN Council, decided that "Conseil" is correct. By the way I consider it reassuring that no attempt was made to translate the acronym; that the OECD is

IMAGE UNAVAILABLE FOR COPYRIGHT REASONS

Isidor Rabi, catalyst in the creation of CERN, with his idea made manifest as the synchro-cyclotron building is constructed. In the background are the Jura Mountains.

initially there was no unanimity at all about the form it should take. Some of the older physicists (Chadwick and G.P. Thomson, for example) thought mainly in terms of collaboration between existing centres. Others (Bohr, Kramers) advocated that several centres should be created, connected or at least in close contact with existing ones, and that stipends should be made available for visitors to such centres. The greatest singleness of purpose was shown by those such as Amaldi and Auger who wanted to set up a new institution around a very large accelerator, and they were soon joined by a few others (Kowarski, Bakker). Their ideas prevailed when on 15 February 1952 a convention was signed. These controversies, which at times threatened to frustrate all plans, and their solution are described in detail in the book.

The role of the various national governments is carefully investigated as well. In the received history this role is belittled and it is claimed that CERN succeeded because the entire project remained in the hands of scientists. This assertion cannot be maintained, but the relation between scientists and governments was a peculiar one; I shall come back to that. Moreover,

called OCDE in France, OESO in the Netherlands and so on shows that that body has not entirely escaped parochialism in the participating countries.

Once the convention had been signed two important issues had to be decided at short notice: the choice of a site and the choice of a Director General. Again there were important differences of opinion. The choice of Felix Bloch — a Nobel prize winner and a brilliant scientist, but with an aversion to management — turned out to be rather unfortunate. He was succeeded by Cornelis Bakker, at first objected to by some because he was considered to be "a competent, though not a really outstanding physicist". As others pointed out, however, Bakker had "both the organizational and administrative skills required for this task, and an intimate knowledge of what was involved in designing and building an accelerator".

In a final chapter, "The How and Why of the Birth of CERN", John Krige and Dominique Pestre summarize the story and draw some conclusions. My remaining remarks are largely based on that chapter, but the emphasis may be somewhat different. CERN was successful partly because of the drive of its

originators, but partly it was a matter of good luck. While the organization was in its birth throes it became increasingly clear that during the following decades the real frontier of fundamental science would shift more and more towards high-energy physics and that would make large accelerators indispensable. It was equally fortunate that during that period a new technique — strong focusing — made its appearance. It promised to make accelerators much more efficient, and while it also made designing and building them perhaps more of a risk the task certainly became more of a challenge.

As to the relations with national governments, the authors point out that although the governments were by no means passive, they reacted to events rather than taking the initiative. Plans were mainly drawn up by the scientists and discussed at Council meetings. After agreement had been reached, the government representatives communicated the proposals back home, trying to get them approved. As the authors put it, these representatives often acted more like representatives of CERN trying to influence their governments, than like representatives of their governments trying to influence CERN. Why did the national governments accept this situation? On the one hand research at CERN was not expected to lead to any results of

direct industrial, commercial or military importance, so governments did not feel obliged to exert rigorous control. On the other hand a high level of competence in many branches of technology as well as in high-energy physics properly speaking was seen to be potentially very useful in the long run; therefore they were willing to give support. The book also draws attention to the remarkable stability of the Council, a feature that certainly contributed to CERN's efficiency.

On one matter I disagree with the authors. On page 534 they state: "Hence we find a number of provisions inserted in the convention early in 1953 which (retrospectively) appear rather unusual — no national quotas in the recruitment of personnel . . . no attempt to correlate the value of industrial contracts awarded with a country's level of contribution to the CERN budget . . . no right of veto in the Council including the vote on the budget . . .". I do not find these provisions unusual at all. I am even convinced that it has been the bane of many international organizations that they have tried to stipulate in detail the rights of each of the participants. At the end of page 535 I read "A decade later when scientists connected with CERN tried to pull it off again, tried to set up a comparable body, The European Space Research Organization (ESRO), the member states were on their guard, and laid down a number of restrictions in advance — a fixed total budget for the first eight years, a 'just return' on contracts etc. Governments also learn". Unless this is meant ironically — and I don't think it is — I disagree. Even though I have to admit that the commercial aspects of space research are more important than those of high-energy physics, I would have been inclined to write "Unfortunately the success of CERN was insufficient to teach governments a lesson".

This is a very valuable book. It gives a clear, readable, well-documented and — as far as I have been able to ascertain — accurate and impartial account of the history of a remarkable organization. The authors do not indulge in speculation and do not really try to characterize the dramatis personae, but because actions and opinions are described in detail the protagonists become far more real than the dedicated and unselfish heroes of the traditional myth.

The history of CERN is interesting for its own sake but the importance of this book goes beyond that. All scientists involved in management and all managers involved in science will stand to profit by studying this lesson of the past. ☐

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Trouble in the far north

Bernard Stonehouse

The Fourth World: The Heritage of the Arctic and its Destruction. By Sam Hall. Bodley Head, London/Knopf, New York: 1987. Pp. 240. £12.95, \$17.95.

This book is a denunciation of the white man's influence on Arctic peoples, cultures and landscapes. The author, a journalist and reporter, has travelled widely in the north and has read a great deal about it. His message is clear. Southerners, from seventeenth-century whalers to twentieth-century oilmen, have invaded and exploited the north. The result has been a cumulative destruction of northern cultures from Alaska to Svalbard and across Asia. Diseases, religion, alcohol, money, greed, land-grabbing and bureaucratic insensitivity have all made their contributions. There is nothing in the present situation to suggest that it is improving. In Sam Hall's eyes the north is in a mess, southerners are unequivocally to blame, and it is time that those responsible did something to try and put things straight.

The book is in three parts. The first contains a brief history showing how Eskimos (now Inuit) spread from Siberia to North America and Greenland only a few thousand years ago, developing their skills and cultures for dealing with the Arctic as they went. Part 2 tells of the explorers, missionaries, whalers, hunters and traders who headed north with a variety of motives, and the consequences of their depredations on both Inuit and Indian. Part 3 describes how new southern threats, from war games to wind-blown pollution, are maintaining the pressures, how unsettled and miserable indigenous folk have become, and how new political and social groupings are arising among them to counter southern hegemony. A selected bibliography and index round off the book.

Hall's views are not new but they are timely and valid. There are mistakes and purple passages that almost convinced me to give up at first reading, but I'm glad I didn't. *The Fourth World* is an up-to-date exposition of the problems facing Arctic and sub-Arctic humanity, written vigorously, unpretentiously and with conviction, and it is worth reading by anyone who cares about the Arctic, culture conflicts or misruled minorities. It is not without bias — but let those who feel misrepresented write an equally vigorous defence of their viewpoint. ☐

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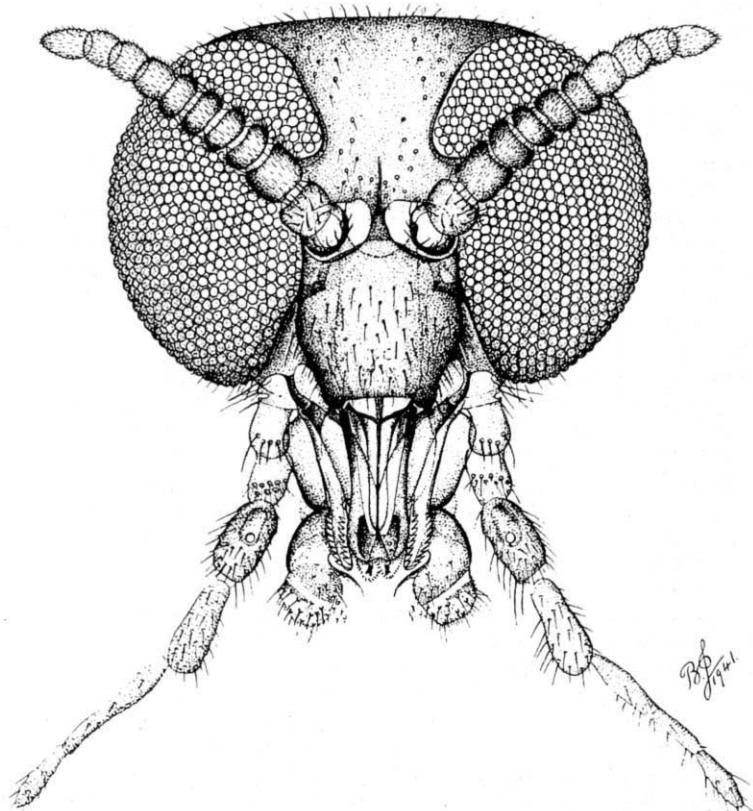
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Eye for detail — anterior view of the head of a blackfly, *Simulium (Wilhelmia) sp.*, drawn by the late Boris Jobling. The picture is reproduced from *Anatomical Drawings of Biting Flies*, which has been published by the British Museum (Natural History) both as a working anatomical atlas and to draw attention to the Jobling collection of specimens and drawings in the Wellcome Museum of Medical Science, London. The book was prepared for publication by David J. Lewis and costs £9.95.

Fantasy and reality

Michael Spencer

Past, Present, and Future. By Isaac Asimov. *Prometheus*, Buffalo, New York: 1987. Pp. 374. \$19.95.

ISAAC ASIMOV has published more than 350 books, hundreds of short stories and much else besides. He turns out at least 500,000 words a year and has never suffered from writer's block.

Follow that, as they say. Those who know Asimov best as a prolific science-fiction writer, and as a compulsive popularizer of real science, will find here that he is also a master of the supposedly lost literary art of the elegant one-topic essay. The 66 pieces in this collection first appeared in a wide selection of popular or technical magazines, mostly in the United States. They range from history to prophecy, from the workings of the brain to the manipulation of genes. They also provide revealing insights into how Asimov works and what constitutes his basic philosophy.

In *Write, Write, Write* he lays down principles that should (but don't) guide all authors of scientific papers, let alone those hoping to reach a wider audience.

Understand the mechanics of the language, including grammar; never use a long word if a short one will do; avoid a complex sentence if a simple one will get the thought across. Asimov's devotion to a "spare" style leads him to eschew figures of speech or literary allusions if he can possibly do without. In fiction, he keeps all his actors on a bare stage — not for him the discursive filling-in of background or the rounding out of character description with non-essential detail. A true son of the twentieth century.

Asimov's obsession with paring everything down to the bare bones is all of a piece with his passionate devotion to logic and rationalism; his supreme hero in the world of fiction is Sherlock Holmes. In this strength, however, lies a seed of weakness. Although he issues some sombre warnings about the future, Asimov cannot bring himself to believe that human beings will not (given time, and enough education in science) become as clear-thinking as himself. The real world sometimes fades from view behind a mist of scientific optimism.

This tendency is most apparent in Asimov's recurrent references to the world population problem. In *The Useful Ivory Tower* (a plea for research to continue unchecked, whatever the consequences) he points out that medical

science has reduced the death rate but not the birth rate, so that the population of the world is rising alarmingly. This is not, he says, the fault of science but of "strong traditions against lowering the birth rate". He calls this "insanity", and in another essay (*Society in the Future*) looks forward to a time when human beings will collectively "reorganize their way of life" to lower the birth rate the world over. All will then be well, says Asimov, if we can avoid the twin danger of blowing the planet to bits.

This line of thought looks suspiciously naive because it begs so many questions. Whose population is rising? Not that of the industrialized countries, the leaders of which are so ready to lecture the Third World on its fecklessness. Why is population rising only in the Third World? Could it be not antique prejudice but the need of poor families to have extra pairs of hands in order to survive? Why are so many Third World countries unable to rise out of poverty? Could it be relevant that the United States has only a twentieth of the world's population but accounts for one-third of its consumption of resources? And so on.

One may ask whether it matters that Asimov occasionally slips into simplistic euphoria about the future. After all, he offers his predictions with suitable caveats about their unreliability. The fact is, however, that he takes himself quite seriously and has millions of readers all over the world who may do the same. Is there a danger that Asimov's hopes may be seized upon by policy-makers while his warnings are ignored?

I think there is, because it can only distract attention from our present problems to imply that we can somehow leapfrog over them into a technological paradise: computers and robots to do the work, creative leisure for all, the colonization of space and all the rest. What happened to greed, exploitation and the oppression of the weak by the powerful? In a few of his more sober essays Asimov seems to acknowledge the difficulty, but optimism nearly always triumphs over doubt.

There is no shortage of warning signs. President Reagan's infatuation with the Strategic Defense Initiative is surely a classic example of the danger of embracing futuristic illusions, of confusing fantasy with reality. Asimov angrily denounces this (in *The Case Against 'Star Wars'*) as "bad science fiction"; he sees the scheme as an affront to scientific rationality, if only because of the half-baked way in which it was conceived. Can he, though, deny all responsibility for helping to mould a culture in which so many ordinary Americans see nothing wrong with the idea? □

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Future informative

C. J. S. Clarke

Mind Tools: The Five Levels of Mathematical Reality. By Rudy Rucker. Houghton Mifflin: 1987. Pp. 328. \$17.95.

POPULARIZING pure mathematics for the man in the street (or at least the humanities graduate on the campus) is a task which daunts most mathematicians, but one to which Rudy Rucker rises with aplomb. The structure of his latest book comes from a thesis that mathematics is based on five archetypal ideas: number, space, logic, infinity and information, more or less in the sense of Shannon's "information theory". According to Rucker, we are now entering an era dominated by the fifth archetype, and his aim is to give an account of the other four in the light of information.

The result is a highly individual perspective on mathematics. The remaining unclimbed peaks (Riemann hypothesis, Poincaré conjecture, Goldbach conjecture) are passed over in favour of what is thought to be the area of the future: discrete mathematics (including an implicitly constructive approach to the reals) and the description of complexity. In places this gives rise to a rather cavalier attitude to the traditional approach — for example, in identifying the infinite

numbers of non-standard analysis with the infinite ordinals of conventional set theory.

The start is low-key, wandering through the integers in an apparently effortless conversational style. In this area there are no surprises and the book at first comes out second best to others. (For modern pythagoreanism — the use of numbers as an allegory in metaphysical arguments — nothing can touch the originality of Arthur M. Young's *Geometry of Meaning*, and for the personalities of individual numbers *Les Nombres Remarquables* by Le Lionnais and Brette is indispensable). But the pace picks up through tilings and fractals, covering much else *en route*, and reaches a climax on the home straight to computational complexity and Chaitin's theorem.

The reader is left with a picture of the Universe as a vast computer, in which the human brain is a very finite subsystem with abilities strictly limited by a bound that can be calculated. Although I am not convinced that the classical Turing machine provides the right paradigm for a discrete universe (quantum theory still has something to tell us about how to combine fuzziness and discreteness), I still recommend all the bio-computers reading this review to include Rucker's book in their input data. □

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Social business

Alan Dixon

Primate Societies. Edited by Barbara B. Smuts, Dorothy L. Cheney, Robert M. Seyfarth, Richard W. Wrangham and Thomas T. Struhsaker. University of Chicago Press: 1987. Pp. 577. Hbk \$60; pbk \$27.50. To be published in Britain on 30 July, hbk £47.95; pbk £19.95.

BECAUSE of the inherent drawbacks of the genre, most edited volumes on primate biology fail to live up to the promise of their titles. *Primate Societies* is an exception. Although the book has five editors and 46 contributors, it is a superb synthesis of knowledge about the social lives of non-human primates.

The five sections deal with the evolution of social diversity, socioecology, group life, communication and intelligence, and future prospects for research. Detailed descriptions of social organization in prosimians, monkeys and apes are provided in the first 14 chapters, which contain comprehensive tables of data on population densities, group sizes, ranging patterns, and dietary and reproductive habits. Field studies continue to show how

variable primate social structures are, even within a single species. Bearder describes the complexity of social communication in nocturnal prosimians. The extraordinary tarsier, usually regarded as a non-gregarious species, is now known to live in pairs under some circumstances. Anne Wilson Goldizen presents surprising evidence for polyandrous social organization in some callitrichids — primatologists have long assumed that marmosets and tamarins live in monogamous family groups. These new findings could explain unusual features of callitrichid reproduction, such as large relative testis size in some species.

Marina Cords surveys the social organization of forest-living guenons (*Cercopithecus* spp.) and of the terrestrial patas monkey. The traditional view that these monkeys live in one-male units must now take into account the observation that additional males sometimes enter groups and mate with the resident females. Among the colobine monkeys an influx of new males or "group takeover" may be associated with infanticide of some, or all, of the existing offspring. When this phenomenon was first reported in langurs, many primatologists interpreted it as aberrant behaviour in overcrowded populations. However, Struhsaker and Leland

review the evidence and present a strong case for infanticide as a reproductive strategy in male colobines.

Rodman and Mitani describe alternative reproductive strategies employed by male orang-utans. Adult males are typically solitary and possess marked secondary sexual characteristics (cheek flanges and a laryngeal sac). There is, however, a second class of smaller males which do not have these features; these males are reproductively active, but they avoid aggressive contact with the bigger, territorial males.

The socioecology section makes it clear that relationships between ecology and social organization in primates are much less simple than was believed 20 years ago. Indeed it is very hard to obtain measurements of variables, such as predation pressures, which may have influenced the evolution of social organization. More progress has been made in understanding the dynamics of group life and how competitive or cooperative interactions affect the reproductive success of group members. The third part of the book contains 11 chapters on this theme, and I particularly enjoyed the reviews on patterns of sexual activity, sexual competition and mate choice by Hrdy, Whitten and Smuts.

The final two sections are much shorter and deal with communicatory biology and prospects for research on primate social organization. There is a very good contribution by Seyfarth on vocal communication, but only one short chapter is devoted to olfaction and vision. Additional coverage of these areas would have been valuable.

Field workers have made many contributions to conserving primates as well as studying their behaviour and ecology. The penultimate chapter by Mittermeier and Cheney is a sad reminder that more than 30 of the 51 extant primate genera contain endangered species and that human overpopulation results in the destruction of 10–20 million hectares of rain forest each year. Apart from the great importance of protecting these animals for their own sake, without habitat conservation and captive breeding programmes most biomedical research involving non-human primates has no future. □

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• Newly published by Springer-Verlag is *Primates: The Road to Self-Sustaining Populations*. The book (a large one, of 1,044 pages) is the proceedings of the conference of the same name held in San Diego in June 1985. It contains 74 contributions on various aspects of the main topic, principally case-histories of conservation in the wild, captive breeding and primate health. The collection is edited by Kurt Benirschke and costs DM 198.

Making sense of the Universe

A. Rupert Hall

Isaac Newton's Principia Mathematica was first published 300 years ago. The book's impact was immediate, and its appearance marked the advent of modern science.

"I HAVE at length brought your Book to an end, and hope it will please you". Edmond Halley wrote to Isaac Newton on 5 July 1687, at the end of some 13 months of editorial effort during which Halley had more than once smoothed the temper of the easily ruffled Newton. The author received 20 free copies; Halley also sent him another 40 to be sold for Halley's benefit at five shillings a copy in sheets (the price bound in leather was nine shillings). The best estimate is that 400 copies of the book were printed, at Halley's expense, the Royal Society being without funds at the time. The Society had received early accounts of Newton's work in mechanics and then Book I of the *Philosophiæ Naturalis Principia Mathematica* with unprecedented enthusiasm; only the astronomer John Flamsteed looked a little sourly on Newton's achievement and, worse, the experimenter Robert Hooke had "some pretensions upon the invention of the rule of the decrease of Gravity, being reciprocally as the squares of the distances from the Center". Others attached the epithet 'incomparable' to the *Principia* from the first.

Hooke's challenge — cause of the lasting breach between the two men that postponed the publication of Newton's *Opticks* to 1704 — provoked Newton into relating to Halley the history of celestial mechanics (as Newton saw it). In 1666 Newton had discovered the inverse-square law, as well as its relation to the r^3/T^2 of Kepler's Third Law of planetary motion and the v^2/r of circular acceleration, first published by Huygens later. Besides deducing "that the forces which keep the Planets in their Orbs must [be] reciprocally as the squares of their distances from the centers about which they revolve" (his own later words, pretty well confirmed by the extant documents), from the length of the pendulum beating seconds Newton corrected Galileo's estimate of the acceleration due to gravity on Earth. He was thereby able to compare "the force requisite to keep the Moon in her Orb with the force of gravity at the surface of the Earth, & found them answer pretty nearly". (The agreement would have been better had Newton not used Galileo's underestimate of the Earth's diameter.)

Nevertheless, in 1666 Newton was not yet thinking of universal gravitation nor yet of motion in planetary ellipses. It is now

clear that within less than a decade before the publication of the *Principia* Newton still supposed the planetary motions to be caused by some sort of aetherial mechanism; moreover, it was thanks to the brief correspondence with Newton, opened in 1679 by Hooke (then Secretary of the Royal Society), that Newton discovered the supreme importance of Kepler's *Second Law*, the law of equal areas — not

IMAGE
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REASONS

Isaac Newton — a mind for revolution.

only did this law describe the variation in a planet's orbital speed, it described *with complete generality* the motion of a body about a centre towards which it is accelerated. There lay the key to proving the consistency between the elliptical orbit and the inverse-square law. It is demonstrated in Proposition I of the *Principia*.

The amazing fact is that, having established these crucial relations in January 1680, Newton "put the calculations aside" until Edmond Halley (perhaps the finest English mathematician then alive, after Newton) rode to Cambridge to ask Newton to make astronomical sense of the inverse-square law. Within three months the barest sketch of a new mechanics, not

forgetting comets and projectiles, was in Halley's hands and presented to the Royal Society. The work on tides, on the flow of liquids, on the particle theory of light, on wave-motion and the speed of sound was (with much else) all developed later, mostly during 1685. Newton's later claim that 'the book of the Principles' was written in 18 months was not far out, such was the power and speed of his mathematical invention.

The book changed and grew as Newton wrote it. Originally it was to have been one unbroken series of propositions, like the first sketch presented to the Royal Society in November 1684. Then, as his treatment of rational fluid mechanics expanded to include the wonderful theory of wave-motion constructed *ab origine* and the analysis of the rotation of vortices, Newton separated this part of his work into a Book II, limiting the first Book to the basic theory of orbital motion. He had also decided to illustrate in some detail, but in an accessible manner, the agreement of his celestial mechanics with the revolution of the Moon, planets and satellites, the precession of the equinoxes, the trajectories of comets and the tides. The compilation of extensive data cost him great efforts and the friendship of Flamsteed. In his anger against Hooke, in the summer of 1686 Newton hastily resolved to eliminate Book III altogether; partly under Halley's persuasion he in fact re-composed it in a more mathematical form. The original Book III was printed separately after Newton's death as *The System of the World*. Although the early sections of the *Principia* have been of the greatest interest to mathematicians, Book III has always presented the newtonian universe to the more general reader; Halley was right to insist upon its indispensability.

Throughout his later life, darkened by the dispute with Leibniz over who had discovered the calculus, Newton claimed that not only had he outlined his calculus of infinitesimals in the *Principia* but that the book was made possible by his mastery of such methods. Neither claim is more than half true. From the terse lemma on 'moments' (the term fluxion is not used) no reader could have re-created the techniques developed by Newton since 1664. And only one proposition in the *Principia* (on the solid of least resistance) is known to have been demonstrated by a fluxional calculation; hence no proof of it was

Mary Evans

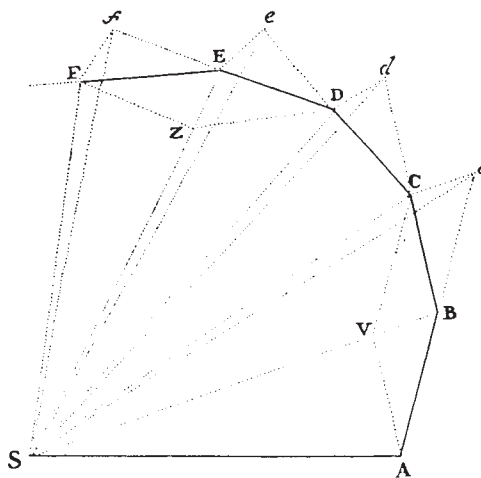
printed in the book. It is certain that otherwise the propositions were devised as they were printed; indeed, they use geometrical infinitesimals with great ingenuity but there is no question of an algorithm or formal calculus. Only Newton's supreme ability as a mathematician made it possible for him to bring to fulfilment such a vast and intricate design as that of the *Principia*; but it was an ability exercised within the framework of seventeenth-century mathematical technique, not at all exploiting the new mathematics that Newton himself, and Leibniz, had created in independence and ambiguity.

John Locke's praise of his friend Mr Newton reminds us that the *Principia* stood out from the first as a book of philosophical as well as mathematical importance. Newton had firm ideas about the transcendental origins and purposes of the Universe, and about the character of human understanding of them. His concepts of time, space and force raised major metaphysical issues. Closely allied to these were the questions implicit in Newton's physical philosophy relating to the homogeneity and simplicity of nature. Newton believed that the invisible world is symmetrical with that which is visible; that the microscopic follows the analogy of the macroscopic; that the science of force and motion which he had established for gravitation might be the model for the elucidation of other classes of phenomena. Though Newton's own tentative investigations of optical and chemical forces proved abortive, his extension of the notion of the fundamental consistency of the Universe, at all levels from the realm of structure to the realm of action, defined a great aspiration for the future.

By 1687 those who had best appreciated Newton's strength as a young mathematician (Barrow, Collins, Sluse) were all dead — although Leibniz, whose first paper on the calculus had been printed three years before, was very much alive. The Cambridge professor was known as the author of some boldly presented experiments on light, and rash proposals for their interpretation. His unexpected appearance as the founder of a new, mathematical system of physics, as the confident opponent of the comfortably established world-view of Descartes, as a new legislator of science, caused international surprise. Those who had rejected Newton's earlier innovations in optics (that is, virtually everyone outside Britain) were unlikely to relish a new incomprehensibility — gravitational force. Both Leibniz and Huygens, first of many during

PROPOSITION I. THEOREM I

The areas which revolving bodies describe by radii drawn to an immovable centre of force do lie in the same immovable planes, and are proportional to the times in which they are described.



The figure and opening text for Proposition I of the Principia, in which Newton demonstrated that Kepler's Second Law holds for all orbits in a force-field. The Principia was first translated into English in 1729, by Andrew Motte; the illustration is taken from Florian Cajori's revision, first published in 1934 by University of California Press.

the next half-century, fabricated neo-cartesian alternatives to newtonian force-mechanics. No one could deny the truth of the mathematical relations that constitute the texture of the *Principia*, but few (outside Britain) could accept the physical ideas giving meaning to these relations. The transition from the (to many) dubious 'newtonian mechanics' of 1687 to the 'classical mechanics' that was the chief intellectual pride of the nineteenth century occupied several generations of mathematicians and philosophers engaged in the clarification, verification and expansion of Newton's ideas and methods.

In the work of revision Newton himself was first, and perhaps foremost. Though the physical philosophy of the second edition of the *Principia* (1713), edited by Roger Cotes and again written in Latin, is unchanged from that of the first, in scientific detail it is a greatly improved book. The whole theory of the Moon, much in the treatment of fluid mechanics and the demonstration of the system of the world were treated more rigorously and exactly. Newton now introduced thoughts about the relation of God and nature, already sketched at the close of *Opticks*. This was a larger edition (711 copies); it made the philosopher Bentley who promoted it a profit of £200, and it was reprinted in the following year at Amsterdam. The changes in the third edition (1726) were of refinement only.

By this time Newton, knight and President of the Royal Society, had become the despot of British science. Some of the young continentals came to admire the great man and submitted to his ideas. The theory of light and colours, experimentally vindicated, was soon accepted every-

where. From 1720, Europe (the Dutch, the Italians, the French and the Germans, in that order) was converted to newtonian physical science, though the conversion was never complete. Leibniz's *vis-viva* (kinetic energy) had to be built into the 'neo-newtonian' system, and the wave-theory of light refused to die. In the algorithm of the calculus Newton had failed, publishing too late, so that the newly formalized mathematical science of Lagrange and Laplace spoke the language that Newton had tried desperately to silence.

The purest vein of newtonianism was extended far into the nineteenth century in the Cambridge Mathematical Tripos; it is not hard to see the newtonian tradition in the two great Cambridge Scots, William Thomson and James Clerk Maxwell. Already in their generation the problems latent in the grand (but never fully consistent) theoretical

structure that Newton had made began to appear, for the long day of the 'mechanical philosophy' was closing and already it had been necessary to rebuild the theory of light and colour. Experimental physics was digging down to new levels of minute structure, and mathematical theory demanded new complexities. Yet the *Principia*, starting from the basic pillars of force, mass and motion, was the platform upon which all this had been built. Not merely for mechanics, but for the whole of physical science (stretching into electricity and chemistry), Newton had provided the coherent set of organizing concepts and the model of experimentation that made subsequent developments possible.

Einstein was to destroy the absoluteness of time and space asserted by Newton as essential foundations of physics, and thereby remove the simple euclidean metric in which Newton and his contemporaries lived. Newton's hard, massy particles have gone, though his picture of a hierarchy of particle orders remains; and it is certain that the mechanical properties of gross bodies are different from those he postulated. For all that, the *ordinary* physical world that we experience as Newton did is still well accounted for by his science. More important, Newton initiated a pattern of investigation that has proved valid from his day to ours; a method that allows flashes of insight to be developed by mathematics and experiment, and that maintains the consistency in physical theory between the largest scale of being and the smallest. □

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A 220-year continuous record of volcanic H_2SO_4 in the Antarctic ice sheet

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Continuous H_2SO_4 profiles observed in snow from several Antarctic locations reveal four major volcanic events of the past two centuries (Agung, Krakatoa, Tambora and another large-scale event not recorded historically). Acid deposition and interhemispheric distribution mechanisms are quantified and then used to obtain an order of magnitude estimate for the H_2SO_4 emissions from these eruptions.

MAJOR explosive eruptions eject large amounts of gas (mainly sulphur dioxide and, to a lesser extent, halogenated acids) and dust (generally silicate ash) into the stratosphere. While ash particles are redeposited at the Earth's surface in the following weeks, SO_2 is slowly converted into sulphuric acid aerosol and in this way can remain in the stratosphere for up to two or three years¹.

The presence of acid droplets in the stratosphere (forming the so-called Junge layer) can reduce atmospheric transmissivity and temperature at the Earth's surface². The relationship between climate and volcanism has been well documented in the wake of recent moderate volcanic events (Mount Agung in 1963, Mount St Helens in 1980 and El Chichon in 1982; refs 3 and 4). These studies have apparently shown a decrease in surface air temperature of up to a few tenths of a degree (Celsius) lasting two or three years. However, this effect is of the same order of magnitude as natural variations, often rendering the identification of climate response very difficult. It would therefore be of great interest to check these results using available data from very powerful past eruptions (for example, Krakatoa and Tambora).

To rate the strength of a volcanic eruption, Lamb⁵ has proposed the dust veil index (DVI) based on atmospheric impact observations following an eruption. Unfortunately no accurate values exist for eruptions before 1880, especially for the Southern Hemisphere. Newhall and Self⁶ make use of a volcanic explosivity index (VEI) as a scale of dissipated energy. At first glance, VEI would appear to be less subjective than DVI. But, because sulphate particles are believed to have a greater climatic effect than ash^{7,8}, the VEI, primarily linked to the amount of solid material injected into the atmosphere, is probably not an adequate parameter for use in investigating the relationship between climate and volcanism.

A scale corresponding specifically to the stratospheric H_2SO_4 load would therefore be extremely valuable. For this, polar-ice cores have been proposed as a means of calibrating stratospheric disturbances of volcanic origin. Hammer^{9,10} has studied in detail the past millennia in Greenland cores and Delmas *et al.*¹¹ the past 100 years in Antarctica.

All glaciological methods for obtaining the stratospheric sulphate loading of volcanic origin are based on the following assumptions: (1) the disturbances affecting the chemical composition of the polar aerosol are recorded in snow layers, (2) the fallout of the stratospheric H_2SO_4 loading, a maximum after cataclysmic volcanic events, is sufficient to affect the polar background tropospheric sulphate level for one to three years. Consequently, H_2SO_4 fallout of volcanic origin is generally detected and identified in ice mainly by the shape and the nature of the peaks which are superimposed over the irregular non-volcanic sulphate background.

The purpose of this report, which extends the Antarctic ice record back over the past 220 yr, that is, further than the well-known eruption of Tambora (1815), is to discuss the two assump-

tions listed above with the aid of numerous sulphate measurements obtained at several Antarctic locations.

Snow layer analysis and dating

Until now, four methods have been used for detecting volcanic H_2SO_4 fallout in polar-ice cores: solid electroconductivity (SEC, also called electroconductometric measurements or ECM)¹², liquid conductivity (LC) of meltwater⁹, acidity ($[\text{H}^+]$)¹¹ and sulphate content ($[\text{SO}_4^{2-}]$)¹¹. All these methods, employed separately, must be used with caution and sometimes give ambiguous results as pointed out by Delmas *et al.*¹¹.

The identification of the pure volcanic H_2SO_4 signals may be improved by a comprehensive study of all major soluble impurities (that is, Na^+ , NH_4^+ , K^+ , Ca^{2+} , Mg^{2+} , H^+ , Cl^- , NO_3^- and SO_4^{2-}). Here we measured major ions by ion chromatography¹³, except for H^+ (by acid titration¹⁴), in samples processed by different methods depending on the location (Table 1). A detailed account of the various stringent analytical conditions (decontamination and test procedures) has been reported elsewhere¹³.

It is then possible to calculate the exact H_2SO_4 content of each snow and ice sample by first subtracting sea-salt sulphate. An additional correction is sometimes required (particularly for the lower part of the Dome C profile) when the H_2SO_4 is known to have interacted with sea-salt particles before deposition¹⁵⁻¹⁷.

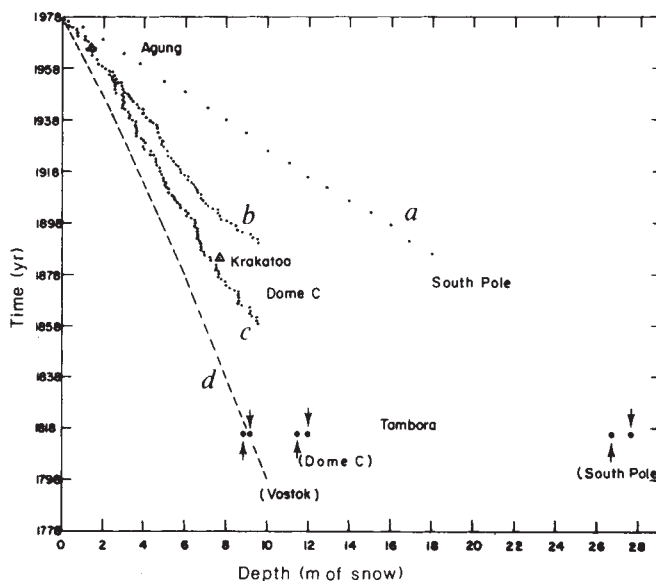


Fig. 1 Timescales of the three cores of this study. *a*, South Pole; *b* and *c*, Dome C; *d*, Vostok station. For Dome C, two datings are possible according to the mean accumulation rate chosen for the calculation. The double volcanic signal of the Tambora years between 11–12 m depth indicates that the timescale *c* is the correct dating (see text).

Table 1 Sampling locations, decontamination procedures, methods and major volcanic events detected at each studied location

Site	Location	Number of samples	Methods	Decontamination procedure	Accumulation rates ($\text{g cm}^{-2} \text{yr}^{-1}$)	Studied time period	Major events detected	Reference
Dome C	74°40' S, 125°10' E	50 240	H ₂ SO ₄ H ₂ SO ₄	a b	3.2 (1955–1978) ¹⁹ 3.06 ± 0.06 (1815–1978)	1959–1969 1760–1959	Agung Krakatoa Tambora Unknown	this work this work
Vostok	78°28' S, 106°48' E	50 Continuous profile (5–12 m) 20	SO ₄ ²⁻ ECM H ₂ SO ₄	a c b	2.25 (1955–85)* 2.00 ± 0.04 2.00 ± 0.04 (1815–1985)	1959–1969 1760–1900 Tambora years	Agung Tambora Unknown	this work (26)
D 55	67°40' S, 137°60' E	80	H ₂ SO ₄	a	7.0 (1955–1981) ³³	1959–1969	Agung	this work
D 80	70° S, 135° E	35	H ₂ SO ₄	b	22.9 (1955–1983) ³³	1959–1969	Agung	this work
D 57	68°11' S, 137°33' E	80	SO ₄ ²⁻	d	18.5 (see text)	Tambora years	Tambora Unknown	21
South Pole	90° S	100 Continuous profile (10–30.5 m) Six other scores (20–29 m)	H ₂ SO ₄ ECM ECM, LC	a c b	8.3 (1955–1978) ²⁹ 8.0 ± 0.2 (1815–1984) 7.7 ± 1.0	1959–1969 1795–1930 Tambora years	Agung Tambora Unknown	16 26, 27 this work and 26, 27
Siple	Antarctic Peninsula 75°55' S, 85°55' W	Continuous profile (0–120 m)	ECM	c	40.0 (20–34) (1800–1984)	1800–1984	Tambora Unknown	20

a, Snow layers collected in pits with the aid of a stainless steel plate.

b, Collection of firm core and subsequent recoring in a cold clean room using a small drill.

c, A flat face is cut parallel to the axis of the core.

d, Thermal probe used to keep only the inner part of ice cores.

Accumulation rates over the last 30 years are determined by radioactive measurements performed on the same sampling: (refs 18, 19, 33).

* Michel Pourchet, personal communication.

In comparison to similar studies in Greenland^{9,10}, one of the first difficulties encountered in the search for volcanic studies in Antarctic precipitation is related to dating (Fig. 1).

At the South Pole, dating accuracy is exceptionally good over nearly all the past 100 yr because the seasonal deuterium variations are particularly well marked and regular for this period^{16,18}. Below ~18 m, the dating of the firm layers is much more uncertain and can be compared to the case of the Dome C where a detailed stratigraphic study¹⁹ has suggested two possible datings (Fig. 1). At this location, the source of our deepest profile, considering the low accumulation rate ($3.1 \text{ g cm}^{-2} \text{yr}^{-1}$, curve c) we would expect the 15.7-m-deep firm core to cover the past two centuries. In such cases the comparison of the H₂SO₄ profiles with the chronology of large volcanic events (for instance the Tambora eruption) is of considerable help in readjusting the dating. This has already been done by Schwander²⁰ to confirm the dating of the Siple ice core and by Zanolini *et al.*²¹ to date a 200-m-deep ice core (at D 57) in an area subject to ice-flow deformations. Finally, for the Vostok station, where no dating was available for the upper firm layers the Tambora signal was used to propose a mean accumulation value over the past 170 yr ($2.0 \text{ g cm}^{-2} \text{yr}^{-1}$).

The 220-yr H₂SO₄ profile at Dome C

The most complete set of results has been obtained at Dome C, where about 220 yr of precipitation have been studied in detail (at least one sample per year). Our discussion will be based on this profile, but the results obtained at other Antarctic locations will be used throughout to back up our arguments. The general trend of the profile, related to atmospheric chemistry changes rather than volcanic phenomena, will not be discussed here.

Using the total β -radioactivity horizons of 1955 and 1965, the shallow snow layers (that is, the past 30 years) can be dated with relative ease at all stations studied (corresponding mean accumulation rates are reported in Table 1).

Delmas and Boutron²³ and more recently Delmas *et al.*¹¹ have

reported records of several volcanic events (in particular Mount Agung and Krakatoa) in their 100-yr sulphate and acidity profiles. Our H₂SO₄ profile at Dome C (Fig. 2) allows examination of a longer time period including the Tambora years (1815). The profile exhibits many events of different magnitudes. We have selected for discussion the nine most significant signals. Among them, four stand out very clearly (peaks 1, 8, 9 and 4).

A simultaneous increase of total β -radioactivity and sulphuric acid content is observed in our samples corresponding to 1964–65 snow layers. Peak 1 is therefore clearly linked to the Agung eruption as already widely explained elsewhere¹¹.

We then tried to identify the other events. From historical records, we selected major volcanic events of hemispheric concern (VEI ≥ 4 or DVI ≥ 400) over the past two centuries (see Fig. 2), assuming that the contribution from volcanoes north of 20° N is minor in the Antarctic^{10,11}. We were unable to find any definite relation between the spikes of our H₂SO₄ profile and the eruptions of the VEI chronology, except for the eruptions also listed in the DVI compilation. The only two eruptions with VEI equal to 4 to be clearly recorded in the H₂SO₄ profile are Agung (peak 1) and Nilahue (peak 2, see below).

To compare the strength of these events, instead of using the highest level of the signals, we calculated the deposition flux of H₂SO₄ (Φ) from the area of the peak from which was subtracted the area corresponding to the non-volcanic background. The calculated values are reported in Fig. 2 for each of the peaks discussed.

Considering the dating obtained for each event (calculated according to a $3.06 \pm 0.06 \text{ g cm}^{-2} \text{yr}^{-1}$ mean accumulation rate) and the deposition flux, our profile clearly exhibits the records of Mount Agung (peak 1), Santa Maria (peak 3), Krakatoa–Tarawera (peak 4) and Armagura (peak 6) eruptions. Peak 4, with a width of 31 cm, is the longest event recorded in this profile and is assumed to have spanned more than 4 yr. This peak probably corresponds to the sum of the effects of two significant eruptions: Krakatoa (1883) and Tarawera (1886). For

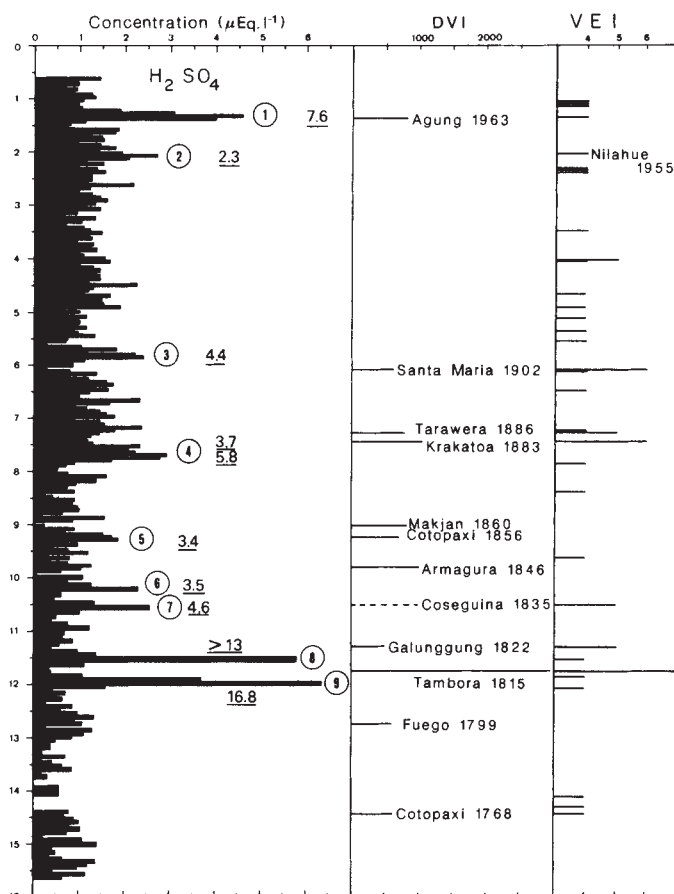


Fig. 2 H_2SO_4 profile obtained at Dome C for the past 200 yr compared with the records of explosive volcanic eruptions south of 20°N . VEI (volcanic explosivity index) values are from ref. 6 and DVI (dust veil index) values are from ref. 5. Major H_2SO_4 spikes, numbered 1 to 9, are of volcanic origin. The corresponding H_2SO_4 amount deposited at Dome C for each of these events is also indicated (underlined values, in kg km^{-2}).

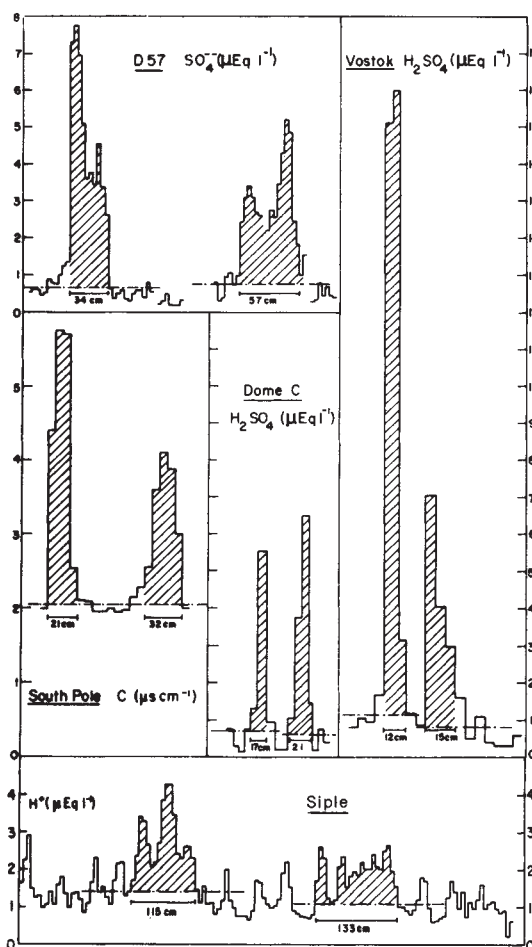


Fig. 3 H_2SO_4 fallout recorded in Antarctic precipitation after the two large eruptions at the beginning of the nineteenth century (one of them is Tambora, 1815, see text). Time from right to left.

peak 5, it is difficult to choose between the eruptions of Makjan (1860) and Cotopaxi (1856).

The weakest of the nine peaks selected (peak 2) is not clearly indicated by the DVI index. We attribute this signal to the eruption of Nilahue (VEI=4), which occurred at a relatively high southern latitude (40°S).

Peak 7 can be attributed to the Coseguina eruption but the observed magnitude in terms of sulphuric acid deposition ($\Phi = 4.6 \text{ kg km}^{-2}$) disagrees with the DVI of 4,000 assigned by Lamb⁵ to this eruption. This possible overestimation pointed out by our data agrees very well with the conclusion of another study²⁴. The Quizapu eruption in 1932, proposed by Hoyt²⁵ as a large event, is not well recorded in our profile.

Peaks 8 and 9, the strongest ones of the profile, could both be attributed to Tambora. The double signal is not an artefact since it has been clearly detected at other Antarctic locations (Fig. 3) by various analytical methods.

We performed ECM down to 30.50 m below the 1984 surface on a core drilled only 50 m away from the pit dug in 1978 at the South Pole and dated by Jouzel *et al.*¹⁸ down to 18 m depth. Our conductometric profile exhibits two large spikes at 27.8 m and 28.6 m depth, respectively. As shown in Fig. 1 (curve *a*) this ECM data strongly supports the idea that one of these two disturbances is linked to the Tambora eruption. A similar pattern is observed on the ECM profile for a 12-m-deep firn core collected at Vostok in 1985 where we also detected two large spikes at 8.85 m and 9.15 m depth²⁶ (see curve *d*, Fig. 1). Finally,

at Siple station, Schwander²⁰ has also found two large spikes in this time period. All these data are consistent and allow us to conclude that one of these two major disturbances is probably a record of the Tambora eruption. The distance between these two maxima of the doublet is (in cm of firn) 45 at Dome C, 80 at South Pole, 30 at Vostok station, 350 at Siple and 220 at D 57 (see Figs 1 and 3). These intervals represent 7, 8, 6, 7 and 8 ± 2 yr, respectively. A first proposal can be made referring to historical records. Indeed in 1822, seven years after the great Tambora event, Galunggung (7°S) erupted. This eruption, estimated at 5 on the VEI index⁶, was indicated by Lamb to have only a DVI of 500 (ref. 5), but this value is uncertain. So peaks 8 and 9 were first attributed to the Galunggung and Tambora eruptions, respectively¹⁵. Greenland profiles¹⁰, for which dating is accurately known (± 1 yr), exhibit only the Tambora signal. A possible but debatable explanation for this difference could be a highly unbalanced distribution of Galunggung debris between the two hemispheres (Galunggung erupted in October, Tambora in April).

Recently, micrometre-sized glasses present in peak 9 have been studied under a high-voltage electron microscope. It was concluded that these glasses did not originate from the Tambora volcano²⁸. Unfortunately, such a study has not been performed on the other high-acid level. Although we cannot be certain until this promising technique has been performed at both depths, we tentatively propose here that peak 8 is linked to the Tambora eruption and peak 9 to an unknown eruption occurring around 1808.

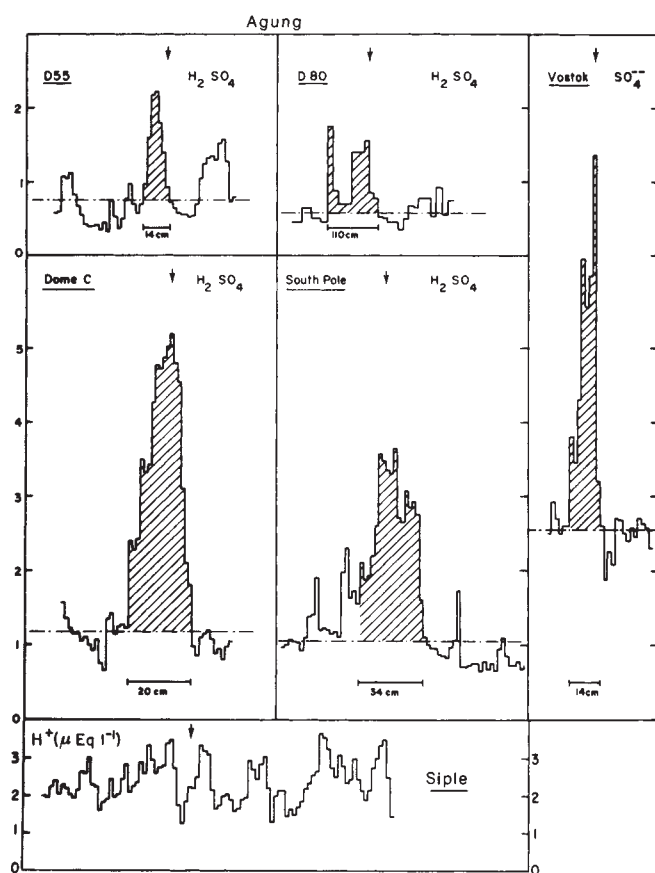


Fig. 4 H_2SO_4 fallout recorded in Antarctic precipitation after the eruption of Agung (1963). The concentration scales are in $\mu\text{Eq l}^{-1}$. D 55 and D 80 are stations located in Terre Adelie (east Antarctica). The date of the Agung fallout at each of the sites is indicated by an arrow. Time from right to left.

In any case our data clearly reveal a second very significant eruption at the beginning of the nineteenth century (at least for the Southern Hemisphere), until now not very well documented in historical records.

Spatial variations of H_2SO_4 deposition flux

As shown in Table 1, data concerning Agung, Tambora and the unknown eruption are available in snow layers deposited at various locations on the Antarctic continent. As previously pointed out^{11,29}, Fig. 4 confirms that the Agung signal is better defined in central areas (Dome C, Vostok and the South Pole) than in more coastal areas (D 80) and is smoothed at Siple station. The same phenomenon is observed for Tambora and the unknown eruption (Fig. 3). This may be due to an enhanced fallout of volcanic debris from the stratosphere to central areas or to an effect related to the dry deposition mechanisms as previously demonstrated for radioelements^{22,30}. We shall examine this spatial variability in detail, generally using the calculated sulphuric acid deposition flux. But this parameter is not available for all the stations. At Vostok (for Agung years) and D 57 (Tambora years) we have only sulphate measurements. At D 57, because H_2SO_4 represents the major contribution of sulphate (90–95%)²¹, we equate sulphate and sulphuric acid. Snow from Vostok, located in the most central areas of east Antarctica, contains significant amounts of Na_2SO_4 (refs 17, 27). Assuming that this neutral sulphate portion remains relatively stable over the Agung time period, we take sulphate variations as being equal to sulphuric acid variations.

For the Tambora years at the South Pole, we assume that liquid conductivity variations are essentially linked to H_2SO_4 content variations (as suggested by recent chemical analyses, S. Kirchner, personal communication). We therefore evaluated the H_2SO_4 flux using the equivalent limit conductivity of sulphuric acid ($0.438 \text{ S cm}^{-2} \text{ mol}^{-1}$). Finally, for Siple we used ECM data

from Schwander²⁰ and calculated the corresponding acid levels with the calibration curve given by Hammer¹².

At D 55, as shown in Fig. 4, we observe an unusual signal for Agung. Indeed, at this location the disturbance spans only one year instead of the two years generally observed. From a comparison of the widths of the Agung signal and the radioactive signal of the 1964–65 snow layers, it seems that half of the volcanic signal is missing, probably due to a reworking of this snow layer by the wind. At Dome C the H_2SO_4 content for peak 8 increases only at the upper extremity of a core section, indicating the probable loss of part of this volcanic signal during the re-coring operation. The reported value for the deposition flux (Fig. 1) therefore probably represents an underestimate and has been disregarded.

For accumulation rates (a) varying from 2.2 to $40.0 \text{ g cm}^{-2} \text{ yr}^{-1}$ (Fig. 5) the flux (Φ in kg km^{-2}) can be expressed in the form:

$$\Phi = 5.5 + 0.4a \quad \text{for Agung} \quad (1)$$

$$\Phi = 27.0 + 1.23a \quad \text{for Tambora (peak 8)} \quad (2)$$

Despite the large error bars for Siple (in relation to the important natural variability of the background at this site), a linear relationship is obtained between Φ and a . The deposition flux appears therefore, to be modulated mainly by the accumulation rate both for Agung and Tambora (peak 8) eruptions.

Assuming that it is possible to extrapolate flux values to accumulation rates smaller than $2.25 \text{ g cm}^{-2} \text{ yr}^{-1}$, a 'dry flux' is obtained. (However, the exact mechanism of impurity deposition in geographical areas of extremely low accumulation is poorly documented and the existence of this 'dry deposition' has not been proved.) A smaller dilution of the 'dry deposition' would therefore explain the larger concentrations observed in such areas.

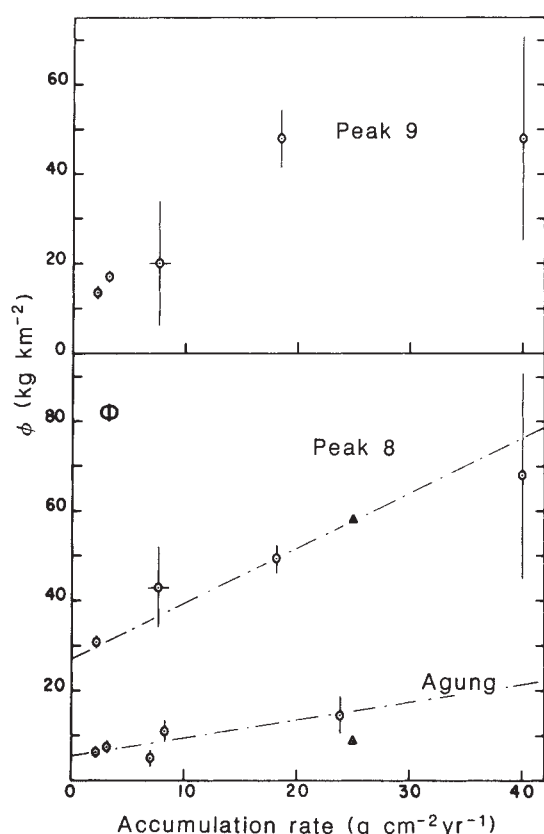


Fig. 5 Amount of volcanic H_2SO_4 deposited in polar snow as a function of accumulation rate (for three major events: Agung, Tambora and the unknown eruption, see text). Open circles: Antarctica (this work), triangles: Greenland (data from ref. 10).

The 'dry H_2SO_4 fallout' represents $\sim 75\%$ of the total deposition of H_2SO_4 at Dome C, 55% at South Pole and 30% at Siple. These fractions are comparable to those obtained with total ^{90}Sr and ^{210}Pb (refs 29, 30).

A linear relationship between Φ and a is not observed for peak 9 (upper part of Fig. 5). Contrary to the case of Agung and Tambora, these data suggest that the deposition of the debris of this eruption is not homogeneous over the Antarctic continent. Indeed it seems that deposition is less in west Antarctica (South Pole and Siple stations), but further study is obviously required before drawing firm conclusions.

Interhemispheric comparison of volcanic records

It may be useful to compare information obtained for the Greenland ice sheet with our data from Antarctica. Indeed events recorded both in the north and the south may be assumed to be of global concern. This is probably the case for large eruptions occurring at low latitudes and dispersing their debris in both hemispheres.

The records over the past two centuries in Greenland^{9,10} and in Antarctica (this work) can be compared. Hammer detected seven events at Crete station (Greenland, accumulation rate: $25 \text{ g cm}^{-2} \text{ yr}^{-1}$) during the 1780–1972 time period, including Agung (8° S), Krakatoa (6° S), Coseguina (13° N) and Tambora (8° S). Besides these four eruptions, located between 20° N and 20° S and recorded in both Greenland and Antarctica ice, Hammer observed the debris of Katmai (1912, 58° N , $\text{VEI} = 6$, $\text{DVI} = 400$), Pogromni (1795, 55° N , $\text{VEI} = 4$, $\text{DVI} = 1,000$) and Lakagigar (1783, 64° N , $\text{VEI} = 4$, $\text{DVI} = 2,300$). The corresponding levels in our Dome C profile, assuming an accumulation rate of $3.06 \text{ g cm}^{-2} \text{ yr}^{-1}$, are 5.4, 13.0 and 13.7 m depth, respectively. These eruptions are not recorded in Antarctica (Fig. 2). Conversely, Hammer did not observe signals from Tarawera (1886, 38° S , $\text{VEI} = 5$, $\text{DVI} = 800$) or Nilahue (1955, 40° S , $\text{VEI} = 4$).

It is therefore clear that moderate eruptions occurring at middle and high latitudes concern only the corresponding hemisphere. Although the Lakagigar eruption can be considered to be more than moderate, it appears to have mainly disturbed the troposphere as suggested by the short duration of the corresponding signal observed by Hammer³¹ at Crete, Camp Century and Dye 3 (1 yr) in Greenland.

Now we examine in more detail the eruptions occurring at low latitudes such as Agung and Tambora. As we know the relationship between flux and accumulation rate for both eruptions, it is possible to compare quantitatively Greenland and Antarctic deposition on the same diagram. In addition to our Antarctic data, the lower part of Fig. 5 shows the corresponding deposition flux observed by Hammer at Crete station. Assuming that Greenland and Antarctica are representative of the Northern and Southern Hemispheres respectively, this comparison could give a good estimate of the interhemispheric distribution of the debris.

While a balanced distribution can be observed for the Tambora eruption, Fig. 5 suggests an approximate two-thirds and one-third distribution of Agung debris between the Southern and Northern Hemispheres respectively. This important difference is consistent with stratospheric sulphate measurements³² which have shown a higher contamination of the southern stratosphere after the Agung event. Because Agung and Tambora both erupted in spring (17 March 1963 and 11 April 1815), this difference reflects the problem of interhemispheric transport and suggests a very different altitude of debris

Table 2 Various parameters of some volcanic eruptions and corresponding temperature changes

Event	Location	Global fallout (Tg) (this work)	Estimates from other workers	Estimates of fine dust (ref. 8) (Tg)	VEI (ref. 6)	DVI (ref. 5)	change ($^\circ\text{C}$)
Agung	8° S	30	>2.94 (ref. 5) 18 (ref. 1) 20 (ref. 10)	1	4	800	-0.35 (refs 24, 35)
Santa Maria	14° N	22	—	—	6	600	
Krakatoa	6° S	30–38	>294 (ref. 35) 55 (ref. 10)	20	6	1,000	-0.35 (refs 24, 35)
Makjan or Cotopaxi	0°	17	—	—	4	750	
Armagura	18° S	17	—	—	—	1,000	
Coseguina	13° N	23	—	—	5	see text	
Tambora (peak 9)	8° S	150	>52.4 (ref. 35) 150 (ref. 10)	150	7	3,000	-0.70 (ref. 5)

injection in the stratosphere. Finally, this comparison between Greenland and Antarctica also shows the strict necessity of possessing information on both hemispheres before proposing a global magnitude scale in terms of the stratospheric H_2SO_4 load.

Estimation of the global H_2SO_4 fallout

The deposition flux of sulphuric acid per km^2 observed in Antarctic snow for a given eruption can be used to assess the corresponding global fallout and hence give the magnitude of the eruption in terms of SO_2 injected initially into the stratosphere.

The total fallout can be calculated from the extrapolation of our Antarctic values by using the global fallout patterns of nuclear test debris as suggested by Hammer¹⁰. In this way, we estimate a global H_2SO_4 fallout of 150 Tg (1 Tg = 10^{12} g) following the Tambora eruption. In the case of the Agung eruption we have applied a hemispheric correction factor (2/3) to take into account the unbalanced distribution of its debris (see preceding section). A global fallout of 30 Tg is proposed for this eruption. By comparing the fallout from these two eruptions observed at Dome C we have also calibrated other eruptions occurring at low latitudes. But because we have only one measurement for the latter eruptions, our magnitude estimates could be out by a factor of 2 when considering a possible reworking of snow layers or an unbalanced hemispheric distribution of the debris.

Our results suggest that the relative amounts of sulphate aerosol produced by the Tambora, Krakatoa and Agung eruptions (all located in Indonesia) were of the order of 5:1:1. These results are slightly different from those proposed by Hammer *et al.* (respectively 7.5:2.5:1.0), but Hammer *et al.*¹⁰ proposed this scale from only one set of data in Crete. Based on these Greenland data alone, their Agung magnitude is probably underestimated. On the other hand, our estimation of Krakatoa fallout is from only one measurement at Dome C as mentioned above and the corresponding Antarctic signal is probably disturbed by the Tarawera debris (see Fig. 2).

Comparing our H_2SO_4 fallout and the amount of fine ash injected into the stratosphere (Table 2), we strongly support the proposition made by Rampino and Self⁸ that the Agung eruption was proportionally richer in SO_2 than either Krakatoa and Tambora. Considering that the mean surface temperature decreased by 0.35 °C and 0.75 °C after the Agung and Tambora eruptions respectively, our data confirm that the climatic effect is primarily linked to SO_2 production.

For the unknown eruption (the twin peak of Tambora), it is more difficult to estimate the magnitude since its latitude is

unknown. But we can assume it is ~45 Tg if located in the high latitudes (>50° S), 90 Tg if located between 20 and 50° S and 135 Tg if occurring in the low latitudes (20° N–20° S). In any case, this eruption was a major event regarding the Southern Hemisphere and may have contributed to the general lowering of atmospheric temperatures detected in the years preceding or following the Tambora eruption (according to the interpretation of the double peak).

Conclusions

This study of Antarctic snow layers covering the relatively well-documented time period of the past two centuries confirms the potential of polar-ice core studies in reconstructing past volcanism in terms of SO_2 injected into the stratosphere. Because detailed records are now available from northern and southern ice caps, the interpretation of the Antarctic H_2SO_4 signals is now more reliable than before.

Although a few results remain unexplained, this work can be considered as a new step in (1) the reconstruction of past explosive volcanism history and (2) the understanding of the spreading and deposition of volcanic debris. In particular, the better quality of records from low-accumulation areas has been shown. This confirms the advantages of using data from the central Antarctic plateau for such studies.

Future measurements from deeper ice layers will extend the data back to times for which the identification of volcanic events will be increasingly difficult. But the glaciochemical method appears to be invaluable in reconstructing the history of stratospheric aerosol loading, one of the most important parameters to be taken into account in climate studies. The polar ice caps, in particular the Antarctic, are privileged observatories of the global aftermath of explosive volcanic eruptions. We suggest introducing a glaciological volcanic index (GVI) to rate the global importance of these eruptions. This index would be more appropriate than VEI for climatic calculations and more accurate than DVI which also includes pure tropospheric effects, particularly for past eruptions with no optical depth measurements, whereas GVI would be primarily linked to the stratospheric aerosol loading. A good assessment of GVI values will require glaciological measurements both in Greenland and Antarctica.

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- Cadle, R. D., Kiang, C. S. & Louis, J. F. *J. geophys. Res.* **81**, 3125–3132 (1976).
- Toon, O. B. & Pollack, J. B. *Stratospheric Aerosol Layer* (ed. Whitten, R. C.) 121–147 (Springer, Berlin, 1982).
- Hansen, J. E., Wang, C. C. & Lacis, A. A. *Science* **199**, 1065–1068 (1978).
- Kelly, P. M. & Sear, C. B. *Nature* **311**, 740–743 (1984).
- Lamb, H. H. *Phil. Trans. R. Soc.* **266**, 425–533 (1970).
- Newhall, C. G. & Self, S. *J. geophys. Res.* **87**, 1231–1238 (1982).
- Newell, R. E. & Deepack, A. *Atmospheric Effects and Potential Climatic Impact* (NASA, Washington DC, 1982).
- Rampino, M. R. & Self, S. *Quat. Res.* **18**, 127–143 (1982).
- Hammer, C. U. *Nature* **270**, 482–486 (1977).
- Hammer, C. U., Clausen, H. B. & Dansgaard, W. *Nature* **288**, 230–235 (1980).
- Delmas, R. J., Legrand, M., Aristarain, A. J. & Zanolini, F. *J. geophys. Res.* **80**, 12901–12920 (1985).
- Hammer, C. U. *J. Glaciol.* **25**, 359–372 (1980).
- Legrand, M., De Angelis, M. & Delmas, R. J. *Analyt. chim. Acta* **156**, 181–192 (1984).
- Legrand, M., Aristarain, A. J. & Delmas, R. J. *Analyt. Chem.* **54**, 1334–1339 (1982).
- Legrand, M. *J. Phys., Paris* **48**, (C1) 77–86 (1987).
- Legrand, M. & Delmas, R. J. *Atmos. Envir.* **18**, 1867–1874 (1984).
- Legrand, M. & Delmas, R. J. *J. geophys. Res.* (submitted).
- Jouzel, J., Merlivat, L., Petit, J. R. & Lorius, C. *J. geophys. Res.* **88**, 2693–2703 (1983).
- Petit, J. R., Jouzel, J., Pourchet, M. & Merlivat, L. *J. geophys. Res.* **87**, 4301–4308 (1982).
- Schwander, J. A. thesis, Univ. Bern (1984).
- Zanolini, F., Delmas, R. J. & Legrand, M. *Ann. Glaciol.* **7**, 70–75 (1985).
- Pourchet, M., Pinglot, F. & Lorius, C. *J. geophys. Res.* **88**, 6013–6020 (1983).
- Delmas, R. J. & Boudron, C. *J. geophys. Res.* **85**, 5645–5649 (1980).
- Self, S., Rampino, M. R. & Barbera, J. J. *J. volcan. geotherm. Res.* **11**, 41–60 (1981).
- Hoyt, D. V. *J. geophys. Res.* **84**, 5018–5028 (1979).
- Legrand, M., Petit, J. R. & Korotkevich, Y. S. *J. Phys., Paris* **48**, (C1) 605–611 (1987).
- Legrand, M. *Thèse d'Etat*, Univ. Scient. et Méd., Grenoble (1985).
- De Angelis, M., Fehrenbach, L., Jehanno, C. & Maurette, M. *Nature* **317**, 52–54 (1985).
- Legrand, M. & Delmas, R. J. *Ann. Glaciol.* **7**, 2–25 (1985).
- Lambert, G., Ardouin, B. & Mesbah-Bendezu, A. in *Precipitation Scavenging, Dry Deposition and Resuspension* (eds Pruppacher, B. *et al.*) 359–372; 1289–1300 (Elsevier, New York, 1983).
- Hammer, C. U. *Jökull* **34**, 51–65 (1984).
- Castleman, A. W., Munkelwitz, H. R., Jr & Manowitz, B. *Tellus* **26**, 222–234 (1974).
- Petere, P., Pinglot, J. F., Pourchet, M. & Reynaud, L. *J. Glaciol.* **32**, 486–500 (1986).
- Herron, M. & Langway, C. C. Jr *J. Glaciol.* **25**, 373–385 (1980).
- Devine, J. D., Sigurdsson, H., Davis, A. N. & Self, S. *J. geophys. Res.* **86**, 6309–6325 (1984).

A new T-cell receptor gene located within the alpha locus and expressed early in T-cell differentiation

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A new T-cell receptor gene lies just 5' to the $J_\alpha C_\alpha$ coding regions. Its placement in this location suggests a novel mechanism for the regulation of expression of one T-cell receptor polypeptide to another during ontogeny. Rearrangement of this locus occurs very early in thymic differentiation and its RNA expression parallels that of the γ -chain in thymic subpopulations, making this a possible candidate for the recently described δ -chain of the T-cell receptor.

THE thymus is the major differentiative organ for T-lymphocytes in higher vertebrates. The regulation of expression and assembly of the T-cell antigen receptor (TCR) genes must relate to the many selective and maturational events which take place in the thymus as T-cell precursors progress through various stages of differentiation. Two distinct types of TCR have thus far been identified: the $\alpha:\beta$ heterodimer found on functional helper and cytotoxic T cells¹⁻³, and the recently described $\gamma:\delta$ heterodimer⁴⁻⁹. Both heterodimers are independently expressed on the cell surface of distinct populations of T cells in association with the monomorphic CD3(T3) polypeptides. Recent studies of the expression of the TCR/CD3 complex show that $\gamma:\delta$ is first detected approximately two days before the appearance of cell-surface $\alpha:\beta$ heterodimer during T-cell ontogeny^{6,7}. In the adult thymus it is found mainly in the least mature cells, the so-called 'double negative' (CD4⁻CD8⁻) population¹⁰. This is a heterogeneous group of very early thymocytes, at least some of which can give rise to all the major cell phenotypes in the thymus and in the peripheral lymphoid organs¹⁰. Whereas $\alpha:\beta$ heterodimers are shown to be necessary and sufficient for T-cell recognition of antigen in association with major histocompatibility complex (MHC) molecules¹¹⁻¹³, the $\gamma:\delta$ receptor bearing cells show MHC-unrestricted cytotoxicity^{8,9}. The genes for the α , β and γ chain have all been isolated and correlated with their specific protein products¹⁴⁻²⁰. We report here the identification of a new T-cell receptor gene, located just 5' to the $J_\alpha C_\alpha$ coding regions, which shows systematic rearrangement in early thymocytes and appears to use V-gene segments of the TCR α -chain family, well before any C_α message or protein is detected. RNA from this locus is expressed at a high level in early thymocytes and adult CD4⁻CD8⁻ cells but not in mature T-cell populations. These data suggest a novel mechanism for the regulation of TCR gene expression during ontogeny as this new locus is deleted when $V_\alpha \rightarrow J_\alpha$ rearrangement occurs. As it is expressed in cell types expressing γ chain messenger RNA and surface $\gamma:\delta$ heterodimers, this TCR gene may encode the δ -chain molecule.

Rearrangement 5' to C_α in fetal thymocytes

In analysing the status of the T-cell receptor α -chain gene in the thymus, we identified a region located ~85-90 kilobases (kb) 5' to C_α showing systematic rearrangements in adult dull CD1⁺, CD4⁻CD8⁻ cells and hybridomas²¹. These rearrangements were first detected by using pulsed-field gel electrophoresis^{22,23} because the $J_\alpha C_\alpha$ locus spans at least 65 kb^{24,25}. To analyse the rearrangement in more detail, we used a fragment (fragment A, Fig. 1) located 60 kb 5' to C_α to isolate cosmid clones extending further 5' as shown in Fig. 1. Fragment A contains the germline J_α region expressed in the TCR α -chain of the T-cell hybridoma, 2B4 (ref. 26) and represents the starting point of the chromosomal walk. A region (pG4.1, Fig. 1) that detects rearrangements in dull CD1/double-negative cells and hybridomas in normal Southern analyses has been identified²¹ approximately 20 kb 5'

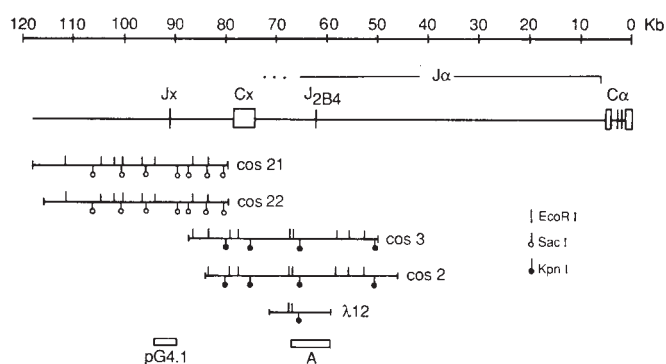


Fig. 1 Genomic organization of the TCR α -chain locus and the location of probes. An oligonucleotide probe specific for the J region of the functional TCR α -chain from the helper hybridoma 2B4 was used to isolate a phage clone (λ 12) containing the genomic coding region of J_α 2B4 (ref. 26) from a B10.A liver genomic library³⁰. The position of J_α 2B4 was mapped to about 65 kb 5' to C_α (unpublished result). An EcoRI fragment (Fragment A) from λ 12 was then used to screen a cosmid library made of BALB/c embryonic DNA⁵⁴. Positive clones cos 2, and cos 3 were isolated and mapped with single and multiple restriction enzyme digestions. The most 5'-end 2.85-kb EcoRI fragment from cos 3 was used to isolate cos 21 and cos 22. Relevant restriction sites are indicated. The DNA fragment used for further analysis was a 4.1-kb EcoRI to SacI subclone (pG4.1); its position is indicated by an open box. The positions of J_α and C_α are determined by restriction enzyme mapping, Southern analysis and DNA sequencing (see also M.I. *et al.*, manuscript in preparation).

to fragment A. We have now extended this analysis to fetal thymocyte DNAs at various days in development to examine the kinetics of this rearrangement process. The results are shown in Fig. 2. These data indicate that rearrangements 5' to the C_α locus appear at least as early as day 14 in fetal thymocytes. Therefore, this locus rearranges at the same time or perhaps just before the TCR β -chain and γ -chain genes^{21,27-29}. Although the rearranged bands become more intense and complex in day 18 and day 19 thymocyte DNA, they diminish in more mature T cells. Consistent with the diminution in mature T cells is the fact that out of nine functional T cells and hybridomas only one helper hybridoma (2B4) retains a single rearrangement of this locus on one chromosome, while the eight others have apparently deleted this region from both chromosomes (ref. 21 and unpublished observations).

To determine the nature of these rearrangements, we isolated a λ -phage containing the rearranged fragment (2B4.Exp) from a 2B4 genomic library³⁰ and compared it with pG4.1. The restriction enzyme maps and sequence analysis of the relevant subclones are shown in Fig. 3. It is clear from these data that there is a J_α -like element (J_x) in pG4.1 and that the rearrange-

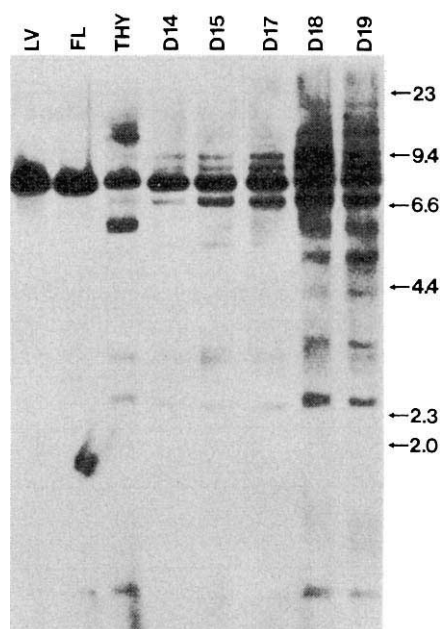


Fig. 2 Southern analysis of 5' α -chain (pG4.1) rearrangements in developing thymocytes. Aliquots of 8 μ g of *Eco*RI-digested genomic DNA from adult liver (LV), day 14 fetal liver (FL), total thymocyte (THY), day 14 (D14) and day 15 (D15) fetal thymocytes of the C57/BL6 strain, and days 17–19 of BALB/c fetal thymocytes were electrophoresed on a 0.8% agarose gel, blotted onto nylon membranes (Gene Screen, NEN Research Products) and probed with pG4.1 DNA (from Fig. 1) which was radiolabelled by hexamer priming⁵⁵. The blot was hybridized in 1 M NaCl, 50 mM sodium phosphate pH 6.5, 50% formamide, 100 μ g ml⁻¹ salmon sperm DNA, 4 $\times$ Denharts and 10% dextran sulphate at 40 °C and washed in 2 $\times$ SSPE and 0.2 $\times$ SSPE at 55 °C. We find no difference in the pattern of hybridization between the C57BL/6 and BALB/c strains with respect to R1 digests. *Hind*III-cut λ size markers are indicated (sizes in kilobases). 20 $\times$ SSPE is 3.6 M NaCl, 0.2 M Na-phosphate pH 7.4 and 0.02 M EDTA.

ment observed in 2B4 is the result of a *VDJ* joining event involving J_α and D elements 5' to J_α (see also Y.C. *et al.*, manuscript in preparation). Sequence analysis of this particular V region shows that it contains all the amino acids that are highly conserved in V_α genes and is in the same gene family as the V_α TA27 of Arden *et al.*³¹, showing 83% homology at the DNA level and somewhat less (70%) amino-acid homology (Fig. 3b). The J_α element has many of the conserved sequences of T-cell receptor and immunoglobulin J regions (as shown in Fig. 4a). It is particularly similar to known J_α sequences both in sequence and in the fact that it ends in a proline residue, a feature of more than half of the known J_α s^{26,31}. Conserved heptamer (AGCTGTG) and nonamer sequences (GGTTTITGG) separated by 13 nucleotides^{32,33} are located 5' to the unrearranged J_α sequence. At the 3' boundary, there is the conserved RNA splicing signal sequence (GTAAGT)³⁴. As also indicated in Fig. 3b, this particular *VDJ* rearrangement results in a frame shift between the conserved sequences of the V -region element and those of the J , a common occurrence in immunoglobulin and T-cell receptor rearrangements, and consistent with the fact that the 2B4 hybridoma bears a functional $\alpha:\beta$ heterodimer³⁵.

***VDJ_x* spliced to novel *C* region in cDNA**

To determine if any transcript containing this *VDJ_x* sequence can be detected, we used the subclone 2B4.Exp (Fig. 3a) as probe for Northern analysis. Under conditions where the J_α element alone could hybridize, we found it to be expressed at $\sim 1/20$ of the level of C_α in 2B4 RNA, but at a higher level in

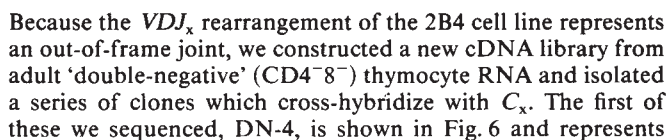
newborn thymocyte and adult double-negative cell RNA (data not shown). We then screened a 2B4 cDNA library ($\sim 200,000$ original clones) and isolated a single clone containing the *VDJ_x* exon described in the previous section spliced to a novel C region (C_x). The C_x coding region exists as a single copy gene in the genome. It is located 13 kb 3' to the J_α gene segment and 70 kb 5' to C_α as indicated in Fig. 1 (data not shown). The genomic organization has been delineated by sequence analysis (M.I. *et al.*, manuscript in preparation). The restriction map of this complementary DNA clone (p λ 12) and its sequence are shown in Fig. 3a and b, respectively. The predicted relative molecular mass (M_r) of this protein would be $\sim 31,000$ (31K). Two potential N -linked glycosylation sites are indicated in the constant-region sequence (Fig. 3b). The C -region coding sequence shares short regions of homology with each of the previously described TCR or immunoglobulin sequences (as shown in Fig. 4b) and is 9–18% homologous at the amino-acid sequence level to any one T-cell receptor sequence. It preserves many of the characteristics of T-cell receptor genes with an extra cysteine past the first domain, presumably for heterodimer formation, and also including a conserved lysine residue in the transmembrane region, a feature of all other T-cell receptor constant regions. It has been suggested that a lysine at this position may be important for interactions with CD3 polypeptides¹⁸. It is interesting in this regard that all three of the CD3 peptides characterized to date have a conserved aspartic acid or glutamic acid residue in their apparent transmembrane regions^{36–38}.

The new T-cell receptor constant region sequence is only weakly homologous (9–18%) to immunoglobulin and other T-cell receptor sequences. The previously reported C_α sequence has a similarly low degree of homology, in contrast to the C_γ and C_β constant region sequences which are much more closely related to immunoglobulins (up to 40% homology in the first domain). It is therefore possible that neither C_x nor C_α form an immunoglobulin-like fold and that some other structure or structures is required for part of the T-cell receptor molecule.

An intriguing similarity between C_x and C_α is that both have relatively short (14–15 amino acids) hydrophobic C termini (counting from the conserved lysine as in Fig. 4a) compared with the corresponding regions of C_γ and C_β (23 and 25 amino acids, respectively) which each have three charged residues near the end of the molecule (C_β ends in 'KKKNS' (single letter code) and C_γ ends in 'NEKKS'). These differences in polypeptide sequence relationships may be important in heterodimer formation and interaction with the CD3 polypeptides. Another feature of the C_x sequence is the similarity in the way C_x conserved sequences are spaced with those of C_α , and the relative disparity with the C_γ and C_β organization (Fig. 4c). In these comparisons, the four T-cell-receptor constant regions are aligned by the conserved cysteine (C), lysine (K), and tryptophan (W) residues and the number of amino acids between are indicated. From this it can be seen that in most cases the distances between the residues are very similar between C_x and C_α and they are very similar between C_γ and C_β . The sole exception to this observation is the distance between the second and third cysteines of C_α which is unusually short. Also interesting is the fact that C_γ and C_β both have a tryptophan 14 amino acids past the first conserved cysteine residue (arrow, Fig. 4b), a feature of all immunoglobulin constant regions, whereas neither C_x nor C_α has a tryptophan at that position or anywhere nearby. As we know that α and β pair with each other, we might suggest x and γ as an equivalent match.

***C_x* expression in immature thymocytes**

To assess the level and distribution of x -chain expression, we prepared a Northern blot with RNA from immature and adult thymocyte populations and probed with C_x , C_γ and C_α cDNA probes (Fig. 5). Both C_x - and C_γ -containing sequences are expressed at a high level in adult double-negative cell RNA



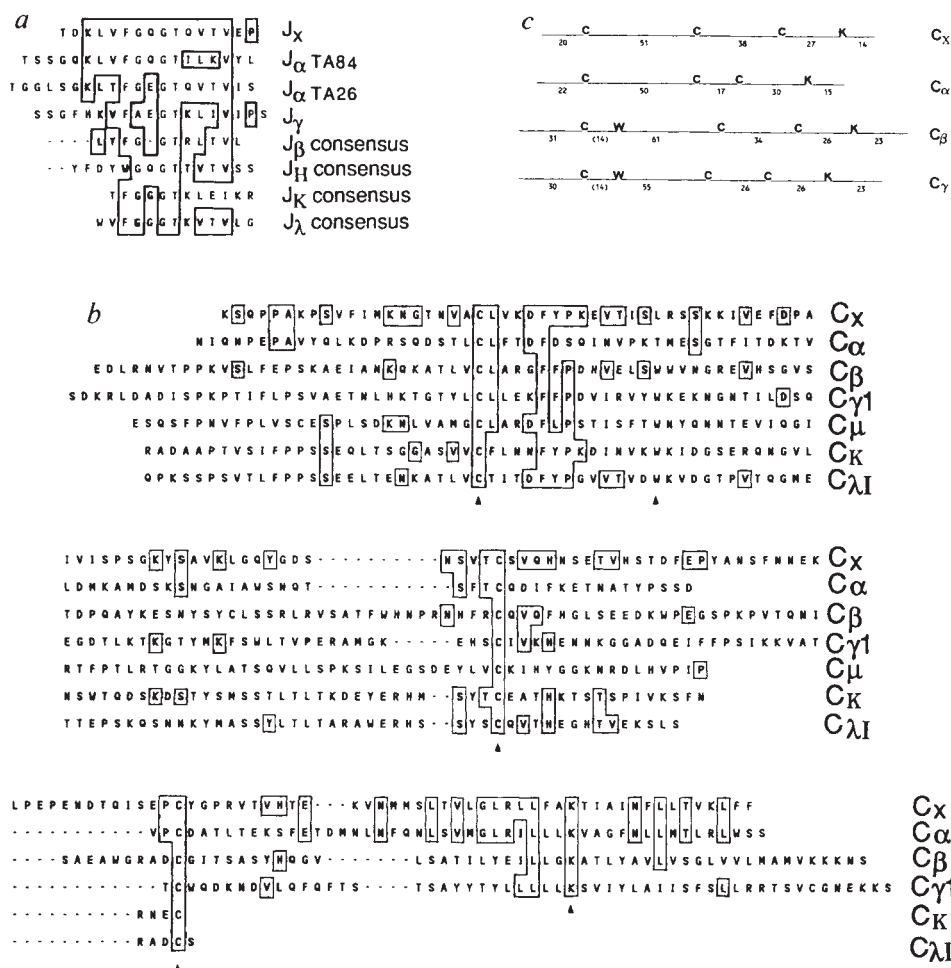


Fig. 4 Similarities of the pA12 *J*- and *C*-region amino-acid sequences to that of murine TCR α - β - and γ -chain and immunoglobulins C_{μ} , C_{κ} and C_{λ} . Similarities are boxed, with gaps represented as dashes. J_{α} TA84 and J_{α} TA26 are from ref. 31. J_{γ} is from ref. 58. The J_{β} consensus sequence is from ref. 59. Immunoglobulin *J* segments are from ref. 60. The mouse C_{α} sequences are from refs 18 and 19, the C_{β} sequence is from ref. 15, the TCR $C_{\gamma1}$ sequence is from refs 17 and 58, and the immunoglobulin sequences from ref. 60. Arrowheads in *b* show the conserved cysteine, tryptophan and lysine residues referred to in the text and in part *c*. The distances between conserved residues in the four different TCR *C* regions discussed. Distances between conserved cysteines and lysines are indicated and the distances between C and W in C_{β} and C_{γ} are in parentheses. C_{β} has an additional cysteine within the first domain which has no homologue in the other T-cell receptor constant regions and is therefore not shown in the figure.

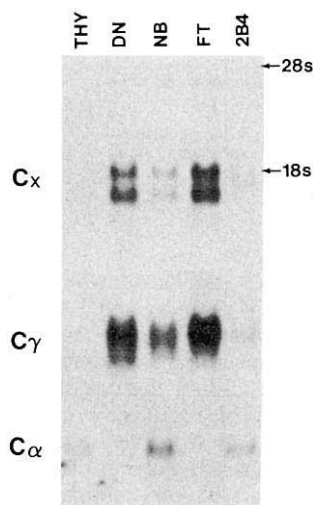


Fig. 5 Northern blot containing 12 μ g each of total RNAs isolated from adult thymus (THY), adult $CD4^{+}8^{-}$ cells (DN), newborn thymus (NB), d15 fetal thymus cultured cells (FT) and 2B4 hybridoma RNA (2B4). Blots were hybridized with either C_{α} , a subclone containing the 3' 0.9-kb *Eco*RI fragment of pA12, C_{γ} or C_{α} , 3.4-kb genomic fragment containing only the first domain exon. The positions of 28S and 18S ribosomal RNA are indicated. Adult thymus, newborn thymus and d15 fetal thymus cultured cells are from the BALB/c strain. Adult $CD4^{+}8^{-}$ cells were isolated from three-week-old BALB/c $\times$ 129 F_1 thymocytes by treating with anti- $CD4$ (GK1.5) monoclonal antibodies (provided by Dr A. Zlotnick) and mouse anti- $CD8.2$ (Cedarlane). FACS analysis showed these cells to be $>99\%$ pure $CD4^{+}8^{-}$. Day 15 fetal thymocyte cells were cultured in IL-4 and PMA for four days³⁹.

an in-frame *VDJ_h* rearrangement. The *C*-region coding sequence is identical to that of pA12, suggesting that the size difference seen between 2B4 and normal cell populations does not involve the coding region. The *V* region of DN-4 has the conserved sequences of a V_{α} gene segment but it is not a member of any previously described gene family. It is, however, 60% homologous at the nucleotide level to the pA12 *V* region, considerably more than the 30% average sequence homology between random V_{α} sequences from different families^{25,31}. Also of note is the fact that this *V* region sequence has a potential

N-linked glycosylation site, as indicated in the figure, bringing the total to three possible *N*-linked sites for this polypeptide.

Relationship to TCR δ

As C_{α} -containing sequences are expressed at high levels in cells where functional γ -protein is found to couple with $CD3$ and another glycoprotein δ , it is of interest to see if C_{α} has the properties of the δ -chain. Functional versions of the sequences we describe here would encode 31K polypeptides with two *N*-linked glycosylated sites in the constant region. A fully gly-

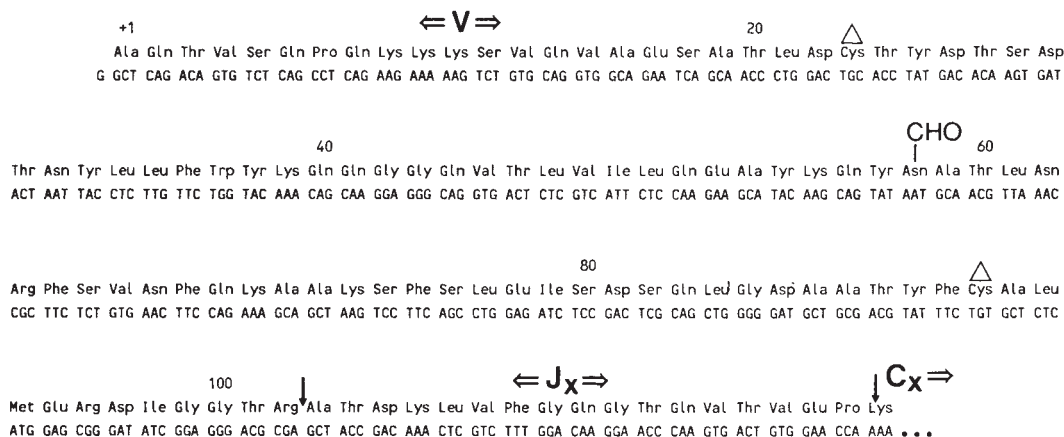


Fig. 6 Sequence of the *VDJ_x* region of the cDNA clone, DN-4. The C region sequence is identical to pA12 (Fig. 3) and is not shown. Cysteine residues and the single potential *N*-linked glycosylation site are indicated as before. Adult double-negative cell RNA, obtained as described in the legend to Fig. 5, was used to make a cDNA library in λ gt10 as described in ref. 30. Clones were screened with a labelled *C_x*-containing fragment. Sequencing of both strands was by the dideoxy-chain termination method⁵⁶.

cosylated form would be ~37–40K, depending on the number of *N*-linked glycosylation sites on the *V* regions. This is considerably smaller than the reported mouse δ -chain protein which is 45K in its glycosylated form and 37K after Endo-F treatment⁶. One possible explanation for this discrepancy is that the structure of the δ -chain protein is unusual, causing it to run slower than its real molecular weight. A second possibility is that post-translational modifications other than *N*-glycosylation increase its molecular weight. Alternatively, the *x* locus may not encode the δ -chain and could thus represent a new class of T-cell receptor.

Regulation of expression

The results presented here and elsewhere²¹ indicate that the $J_x C_x$ locus is rearranged and transcribed before the $J_\alpha C_\alpha$ locus. The evidence for this includes: (1) the detection of J_x rearrangement as early as day 14 in fetal thymocytes (Fig. 2), (2) the inverse relationship between C_x and C_α transcripts in immature versus mature thymocyte populations (Fig. 5), (3) the predominance of J_x rearrangements and the lack of J_α rearrangements in adult dull $CD1^+$, $CD4^8^-$ cells and hybridomas²¹, and (4) the fact that the $J_x C_x$ locus is almost always deleted on both chromosomes of α -chain expressing antigen-specific T-cell lines²¹. The expression of the x-chain before the α -chain, and the tandem arrangement of the two loci, suggests that progressive rearrangements occur on the same chromosome during T-cell differentiation. One possibility is that successful (for example, in-frame) VDJ_x rearrangements might cause a T cell to follow one particular lineage with no further rearrangements on that chromosome. In contrast, a non-functional VDJ_x joining would lead to further $V_\alpha \rightarrow J_\alpha$ rearrangement, with consequent deletion of the x locus and the production of an $\alpha:\beta$ -bearing T cell. The deletion of one locus when another is expressed has precedent in immunoglobulin light chain genes where the κ locus is often deleted in λ producing cells⁵⁰, although in this case, the genes are on different chromosomes. A variation on this model arises from the consideration that the x chain may have to pair with another rearranging T-cell receptor gene (such as γ). If such a partner gene was not successfully rearranged in a given cell then even a productive x locus rearrangement might not be useful and would again lead to its deletion in favor of a $V_\alpha J_\alpha$ event. This possible regulation of expression (or attempted expression) of one T-cell receptor to another using a deletional mechanism has obvious parallels to class switching in immunoglobulin heavy-chain genes^{51,52}, although in that case the original VDJ

rearrangement is preserved. A third possibility is that the rearrangements of the *x* and α loci are unrelated and occur independently.

The tandem arrangement of C_λ and C_α also suggests that differential splicing might take place to allow the expression of a VDJ_λ exon with C_α , analogous to the splicing of a VDJ_H exon of an immunoglobulin heavy chain gene with either C_μ or C_δ^{53} . Such a possibility would have interesting functional implications.

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1. Allison, J. P., MacIntyre, B. W. & Block, D. *J. Immun.* **129**, 2293-2300 (1982).
2. Meuer, S. C. *et al. J. exp. Med.* **157**, 705-719 (1983).
3. Haskins, K. *et al. J. exp. Med.* **157**, 1149-1169 (1983).
4. Brenner, M. B. *et al. Nature* **322**, 145-149 (1986).
5. Lew, A. M. *et al. Science* **234**, 1401-1405 (1986).
6. Pardoll, D. M. *et al. Nature* **326**, 79-81 (1987).
7. Bluestone, J. A., Pardoll, D. M., Sharrow, S. O. & Fowlkes, B. J. *Nature* **326**, 82-84 (1987).
8. Borst, J. *et al. Nature* **325**, 683-688 (1987).
9. Moingeon, P. *et al. Nature* **325**, 723-726 (1987).
10. Fowlkes, B. J., Edison, L., Mathieson, B. J. & Chused, T. M. *J. exp. Med.* **162**, 802-822 (1985).
11. Yague, J. *et al. Cell* **42**, 81-87 (1985).
12. Dembic, Z. *et al. Nature* **320**, 232-238 (1986).
13. Saito, J., Weiss, A., Miller, J., Norcross, M. & Germain, R. *Nature* **325**, 125-130 (1987).
14. Hedrick, S. M., Cohen, D. I., Nielsen, E. A. & Davis, M. M. *Nature* **308**, 149-153 (1984).
15. Hedrick, S. M., Nielsen, E. A., Kavalier, J., Cohen, D. I. & Davis, M. M. *Nature* **308**, 153-158 (1984).
16. Yanagi, Y. *et al. Nature* **308**, 145-149 (1984).
17. Saito, H. *et al. Nature* **309**, 757-762 (1984).
18. Chien, Y. *et al. Nature* **312**, 31-35 (1984).
19. Saito, H. *et al. Nature* **312**, 36-40 (1984).
20. Sim, G. K. *et al. Nature* **312**, 771-775 (1984).
21. Lindsten, T., Fowlkes, B. J., Samelson, L. E., Davis, M. M. & Chien, Y. *J. exp. Med.* (in the press).
22. Schwartz, D. C. & Cantor, C. R. *Cell* **37**, 67-75 (1984).
23. Carle, G. F. & Olson, M. V. *Nucleic Acids Res.* **12**, 5647-5664 (1984).
24. Winoto, A., Mjølness, S. & Hood, L. *Nature* **316**, 832-836 (1985).
25. Hayday, A. C. *et al. Nature* **316**, 828-832 (1985).
26. Becker, D. M. *et al. Nature* **317**, 430-434 (1985).
27. Born, W., Yague, J., Palmer, E., Kappler, J. & Marrack, P. *Proc. natn. Acad. Sci. U.S.A.* **82**, 2925-2929 (1985).
28. Haars, R. *et al. J. exp. Med.* **164**, 1-24 (1986).
29. Born, W., Rathbun, G., Tucker, P., Marrack, P. & Kappler, J. *Science* **234**, 479-482 (1986).
30. Chien, Y., Gascoigne, N. R. J., Kavalier, J., Lee, N. E. & Davis, M. M. *Nature* **309**, 322-326 (1984).
31. Arden, B., Klotz, J., Siu, G. & Hood, L. *Nature* **316**, 783-787 (1985).
32. Early, P., Huang, H., Davis, M., Calame, K. & Hood, L. *Cell* **19**, 981-992 (1980).

33. Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. *Nature* **286**, 676-683 (1983).
34. Breathnach, R. & Chambon, P. A. *Rev. Biochem.* **50**, 349-383 (1981).
35. Samelson, L. E., Germain, R. N. & Schwartz, R. H. *Proc. natn. Acad. Sci. U.S.A.* **80**, 6972-6976 (1983).
36. Van den Elsen, P. *et al. Nature* **312**, 413-418 (1984).
37. Gold, D. P. *et al. Nature* **321**, 431-434 (1986).
38. Krissansen, G. W., Owen, M. J., Verbi, W. & Crumpton, M. J. *EMBO J.* **5**, 1799-1808 (1986).
39. Zlotnik, A. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
40. Snodgrass, H. R., Dembic, Z., Steinmetz, M. & von Boehmer, H. *Nature* **315**, 232-233 (1985).
41. Raulat, D. H., Garman, R. D., Saito, H. & Tonegawa, S. *Nature* **314**, 103-107 (1985).
42. Samelson L. E. *et al. Nature* **315**, 765-768 (1985).
43. Reth, M. G. & Alt, F. W. *Nature* **312**, 418-423 (1984).
44. Kavalier, J., Davis, M. M. & Chien, Y. *Nature* **310**, 421-423 (1984).
45. Siu, G. *et al. Nature* **311**, 344-350 (1984).
46. Rupp, F., Frech, G., Hengartner, H., Zinkernagel, R. M. & Joho, R. *Nature* **321**, 876-878 (1986).
47. Reilly, E. B., Kranz, K. M., Tonegawa, S. & Eisen, H. N. *Nature* **321**, 876-878 (1986).
48. Trauneker, A., Oliveri, F., Allen, N. & Karjalainen, K. *EMBO J.* **5**, 1589-1593 (1986).
49. Zauderer, M., Iwamoto, A. & Mak, T. W. *J. exp. Med.* **163**, 1314-1318 (1986).
50. Heiter, P. A., Kormeyer, S. J., Waldmann, T. A. & Leder, P. *Nature* **290**, 368-372 (1981).
51. Honjo, T. & Kataoka, T. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2140-2144 (1978).
52. Davis, M. M. *et al. Nature* **283**, 733-739 (1980).
53. Blattner, F. R. & Tucker, P. N. *Nature* **307**, 417-422 (1984).
54. Cory, S., Graham, M., Webb, E., Corcoran, L. & Adams, J. *EMBO J.* **4**, 675-681 (1985).
55. Feinberg, A. & Vogelstein, B. *Analyt. Biochem.* **132**, 6-13 (1983).
56. Chen, E. Y. & Seeburg, P. H. *DNA* **4**, 165-170 (1985).
57. Henikoff, S. *Gene* **28**, 351-359 (1984).
58. Hayday, A. C. *et al. Cell* **40**, 259-269 (1985).
59. Gascoigne, N. R. J., Chien, Y., Becker, D. M., Kavalier, J. & Davis, M. M. *Nature* **310**, 387-391 (1984).
60. Kabat, E. A., Wu, T. T., Reid-Miller, M., Perry, H. & Gottesman, K. S. *U.S. Dept. Health and Human Services* (1987).

LETTERS TO NATURE

Neutrino temperatures and fluxes from the LMC supernova

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The observation^{1,2} of neutrinos from the LMC supernova makes possible direct tests of the theory of supernova explosions and of properties of weakly interacting particles. Here we describe a combined analysis of the angular and energy distributions of the events observed in the Kamiokande and the IMB detectors which determines the effective temperatures and fluxes of neutrinos and anti-neutrinos produced by the explosion. Our main result is that a simple model is consistent with the available data and in reasonable agreement with conventional models of supernova explosions. The parameters of the model are: a single temperature, T , of $4.1^{+1.0}_{-0.4}$ MeV, a flux of electron anti-neutrinos of $(0.5^{+0.2}_{-0.35}) \times 10^{10} \text{ cm}^{-2}$ [total energy in $\bar{\nu}_e = (3.0^{+1.7}_{-1.4} \times 10^{52} \text{ erg})$], and a poorly determined flux of 'scattered' (see below) neutrinos = $(0.2-5) \times 10^{10} \text{ cm}^{-2}$. Several statistical tests were used to determine the acceptable range of these parameters. We have also set limits on possible high-temperature fluxes of neutrinos or anti-neutrinos that might result from matter oscillations^{3,4}.

The dominant events in both the Kamiokande (2.1 active kttons) and the IMB (5 active kttons) water detectors are expected^{1,2,5} to be the absorption of electron anti-neutrinos by protons and the scattering of neutrinos of all flavours by electrons. The experimental quantities that we use are the measured energies and angles (with which the detected electron or positron moves relative to the direction of the LMC) of each of the events, together with their quoted 1σ uncertainties. The IMB collaboration, with a threshold just below 20 MeV, observed 8 events with energies between 20 MeV and 44 MeV. The Kamiokande II collaboration (threshold ~ 7 MeV) reported 11 events spread out over 12.4 seconds. The first 8 events occurred within <2 s and were followed by a 7-s gap. The subsequent three events, with lower average energy, could have been generated by a process with different parameters. One might be tempted to speculate that these latter events correspond to scattering of electrons by mu or tau neutrinos (or other exotic particles) with a rest mass of ~ 20 eV (to explain the time delay). This speculation can be ruled out at the 99.7% confidence level because the events in question make relatively large angles with respect to the direction to the LMC ($122^\circ \pm 30^\circ$, $49^\circ \pm 26^\circ$ and $91^\circ \pm 39^\circ$).

The detection of two events in the IMB detector during the gap in the Kamiokande detector reduces the significance of the gap. In a subsequent paper⁶, we show that a thermal spectrum from a cooling neutron star describes well the arrival times as well as the observed energies. Since the neutron star probably cooled significantly by the time (~ 10 s) the last Kamiokande events were observed, we concentrate our single temperature analysis on the first 10 seconds.

We shall consider first solutions in which each type of neutrino (i = electron, muon, tau, and their anti-neutrinos) is described by a thermal spectrum, with an associated temperature, T_i , and a flux, F_i at Earth (measured per cm^2). The rate at which events of a definite energy (E_e) are observed in each detector can be written

$$R(E_e, \{T_i, F_i\}) = \sum_{i,j} F_i N_j \int \int dE'_e dq (d\sigma_j/dE'_e)_i \times \phi_i(q, T_i) \varepsilon(E'_e) g(E_e, E'_e, \sigma'_e)$$

where we denote the various neutrino temperatures and fluxes by $\{T_i, F_i\}$, the energy of the electron (or positron) that is produced by E_e , the neutrino energy by q , the neutrino interaction cross sections by $d\sigma_j/dE$ ($j = 1, 2$ for scattering and absorption), the neutrino spectrum by $\phi \propto q^2 \exp(-q/T)$, the detection efficiency by ε , and the measuring error by g (which we represent in practice by a gaussian). We have linearly interpolated between the reported^{1,2} detection efficiencies to obtain simple representations of the detection efficiencies, ε .

The absorption cross-section is approximately quadratic in energy: $\sigma_{\text{abs}} = 8.9 \times 10^{-42} \text{ cm}^2 (E'/10 \text{ MeV})^2$. The scattering cross-sections are more complicated; in the calculations described below, we have used the full expressions⁷. The following approximation, $(d\sigma_{\text{scat}}/dE')dE' = \text{constant} \times 10^{-44} \text{ cm}^2 (dE'/10 \text{ MeV})$, is useful in interpreting the results. For ν_e -e, $\bar{\nu}_e$ -e and $\bar{\nu}_\mu$ -e scattering, the constant is, respectively, 9, 1.6, 3.9 and 1.3. The total scattering cross-sections are represented, for the energies of interest here, to an accuracy of $\sim 10\%$ by the approximation given above. We have also included contributions from neutrino absorption by oxygen, which, however, is unimportant at the temperatures considered here.

The corresponding formula for the angular distribution is (μ is the cosine of the angle that the recoil electron or positron makes with respect to the direction of the LMC):

$$A(\mu, \{T_i, F_i\}) d\mu = \sum_{i,j} F_i N_j d\mu \int \int d\mu' dq (d\sigma_j/d\mu')_i \times \phi_i(q, T_i) \varepsilon(E_e) S(\mu, \mu') \quad (2)$$

where the smoothing function S represents the uncertainty of the angular measurements, $S \propto \sin \theta' \exp(-\theta_b^2/\Delta(\theta')^2)$ and θ_b is the angle between the measured direction (represented by μ) and the actual direction (represented by μ') in which the electron moves. The absorption events are nearly isotropic: probability

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$P(\mu') d\mu' = d\mu' [1 - 0.10(v/c)\mu']$, where v is the velocity of the produced positron. The scattering cross-section is, on the other hand, strongly forward peaked (see equations (9) and (20) of ref. 7 for the detailed formulae we have used). The IMB detector was biased against backscattering events during the period when the supernova was detected; we included this bias by setting the efficiency for detection equal to 1.0/1.15 for $\mu < 0$.

The functions representing the experimental errors (g in equation (1) and S in equation (2)) are very important since the energy distributions (and interaction cross-sections) of the incoming neutrinos are not flat and the scattering cross-section is strongly forward peaked. Measuring errors shift the apparent energy distribution of events in the IMB detector to significantly higher energies for the inferred anti-neutrino temperatures discussed below. For our preferred temperature (see equation (3a) below), including the measurement errors in energy increases the expected number of events in the IMB detector that are seen to be above 35 MeV by 50% and doubles the total number of events above 40 MeV.

We can at best determine one flux of 'average' scatterers. At the energies considered here, electron, muon or tau neutrinos (or anti-neutrinos) will all scatter electrons in the forward direction. We do not have any way of separating, in a model-independent way, neutrinos and anti-neutrinos of different flavours from their electron neutrino counterparts. For the conventional fluxes and temperatures summarized in Table 1 of ref. 5 and the scattering cross-sections given above, $\sum_i \text{Flux}(i) \sigma_{\text{scatt}}(i) \approx 1.6 \text{ Flux}(\nu_e) \sigma_{\text{scatt}}(\nu_e)$. In what follows, we lump together the fluxes of all neutrino and anti-neutrino scatterers into an 'effective' scattering flux, F_{scatt} defined by $F_{\text{scatt}} \sigma_{\text{scatt}}(\nu_e) \equiv \sum_i \text{Flux}(i) \sigma_{\text{scatt}}(i)$. We expect, but cannot prove, that $F_{\nu_e} \sim 0.5 \times F_{\text{scatt}}$.

Most (but not all) of the information regarding the temperatures is contained in the measured energy distributions; most (but not all) of the information regarding the ratio of ν_{scatt} to $\bar{\nu}_e$ fluxes is contained in the measured angular distributions. The reader can readily appreciate the coupled nature of the problem by considering the following example. The forward peaking observed in the Kamiokande detector (five events within 45° of the LMC direction) suggests an appreciable amount of ν - e scattering, while the relatively isotropic IMB data indicate that, at the higher energies observed in this detector, scattering is not particularly important. The absorption cross-section increases more rapidly with energy than the scattering cross-section (see above); this fact is related to both the relative number of events observed in the two detectors and their different angular distributions. The strongest limits on $F_{\text{scatt}}/F_{\bar{\nu}_e}$ result from the relative isotropy of the IMB events combined with the forward peaking observed in Kamiokande.

We have performed both estimation-of-parameters and goodness-of-fit tests on the data. Maximum likelihood parameters were estimated using a single likelihood function that incorporates the expected energy and angular distributions, as well as the quoted^{1,2} detector efficiencies and measurement errors in each detector. The joint likelihood function also takes into account the absolute number of events in each detector. Use of a joint likelihood function gives parameters that are most consistent with the observations in both detectors. To test whether this consistency is credible, without reference to likelihood, we find the range of parameters allowed by the Kolmogorov-Smirnov (KS) measures of the goodness-of-fit to the observed energies and angles of the individual events. Finally, we have also verified that the maximum likelihood and KS tests are consistent by doing Monte Carlo simulations with the maximum likelihood parameter values. The simulations do indeed produce synthetic data sets that are strongly concentrated in the KS-allowed region.

Figure 1 shows our results for the allowed range of T and $F_{\text{scatt}}/F_{\bar{\nu}_e}$. The star marks the peak of the likelihood function. Solid contours indicate where the likelihood function is 0.1 and

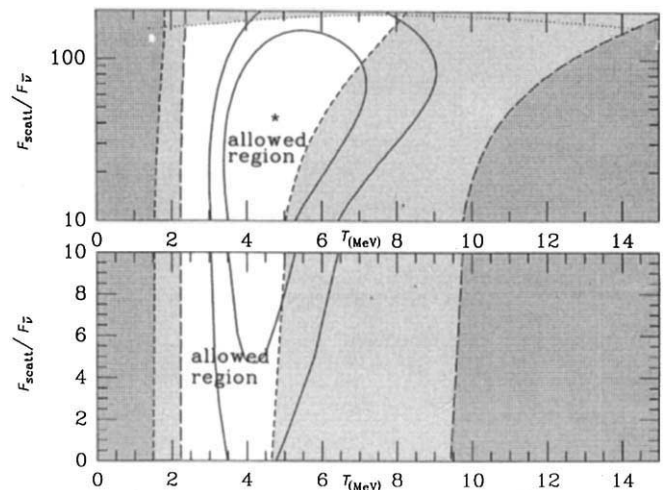


Fig. 1 Allowed and excluded temperatures: the allowed regions are unshaded and the excluded regions are shaded. Contours of the joint likelihood function are indicated by the smooth curves in the $T, F_{\text{scatt}}/F_{\bar{\nu}_e}$ plane. Darker regions are excluded by both detectors. The region outside the long dashed lines is excluded by the energy distribution of the IMB data (at 0.05 significance with a Kolmogorov-Smirnov test). The region outside the short dashed lines is excluded by the energy distribution of the Kamiokande data (at 0.05 significance). The region above the dotted line is excluded by the angular distribution of the Kamiokande data (at 0.05 significance). The overall allowed region is bounded by the upper limits to the temperature from the Kamiokande data, and by the lower limits to the temperature from the IMB data. The solid lines indicate contours of the likelihood function at 10% (inner) and 1% (outer) of its maximal value. While the upper half of the plot is not excluded by the observed data, it is unlikely on the basis of energy considerations (see discussion preceding equation 3(c)). The shadings shown here do not depend upon the relative sensitivity of the detectors.

0.01 of the peak value. Different aspects of the data rule out separate parts of the parameter plane. Darker shades delineate regions that are excluded by data from both experiments. The region outside the short dashed lines is excluded (less than 5% significance level) for the KS measure of the energy distribution of the Kamiokande data. Similarly the region outside the long dashed lines is excluded by the KS measure of the energy distribution of the IMB data. The KS measure of the angular distribution of the Kamiokande data rules out (again with 5% significance) the region above the dotted line. The allowed region (dashed lines) is determined on the high-temperature side by the energy distribution measured in the Kamiokande detector and on the low-temperature side by the IMB energies.

The ratio of scattered events in Kamiokande to absorption events in IMB sets another temperature-dependent limit on $F_{\text{scatt}}/F_{\bar{\nu}_e}$ which rules out high values of $F_{\text{scatt}}/F_{\bar{\nu}_e}$ at low temperatures. Finally, the ratio of the number of absorption events in the two detectors sets a lower limit of 3.7 MeV for the temperature (see Fig. 2a).

The formal maximum of the likelihood function occurs at $T = 4.8$ MeV, $F_{\text{scatt}}/F_{\bar{\nu}_e} = 44$, and $F_{\bar{\nu}_e} = 0.25 \times 10^{10} \text{ cm}^{-2}$. This high value of $F_{\text{scatt}}/F_{\bar{\nu}_e}$ is, however, physically unreasonable; it corresponds to $F_{\text{scatt}} = 11 \times 10^{10} \text{ cm}^{-2}$ and therefore would imply that much more than $0.1 M_\odot$ was radiated in neutrinos (see remarks regarding total energy that precede equation (3c)). The likelihood function is also consistent with smaller values of $F_{\text{scatt}}/F_{\bar{\nu}_e} \leq 10$. For these physically plausible values of the flux ratio, the 'ridge line' (maximum of the likelihood function as T varies at a fixed flux ratio) lies nearly along the constant value

$$T = 4.1 \text{ MeV.} \quad (3a)$$

The corresponding value of $F_{\bar{\nu}_e}$ is $0.5 \times 10^{10} \text{ cm}^{-2}$. If we include the last three events in the Kamiokande data, the maximal

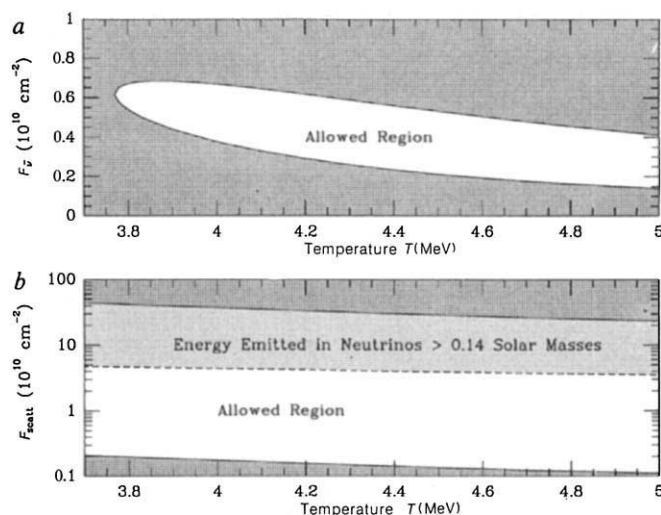


Fig. 2 *a*, Allowed range of anti-neutrino fluxes: electron anti-neutrino fluxes between the solid lines are consistent (5% significance) with the detection of between 3 and 7 anti-neutrino absorption events in Kamiokande and between 6 and 8 absorption events in the IMB. *b*, Allowed range of scattering neutrino fluxes: neutrino fluxes between the solid lines are consistent (5% significance) with the detection of between 1 and 5 scattering events in Kamiokande and 0 to 2 scattering events in IMB. Fluxes above the dashed line correspond to an emitted energy in neutrinos that exceeds the binding energy of a neutron star.

allowed temperature (as determined by the Kamiokande data) is reduced to 3.5 MeV (for $F_{\text{scatt}}/F_{\bar{\nu}_e} < 10$). While this result is consistent with the lower range of the IMB data (see Fig. 1), it reduces significantly the calculated likelihood of the combined data. A better fit to the last three data points can be obtained by assuming that the neutron star is cooling, which naturally leads to a lower temperature producing the later points⁶.

The single temperature model represents a satisfactory fit to the observations. For $T = 4.1$ MeV and $F_{\text{scatt}}/F_{\bar{\nu}_e} = 10$, the probability of obtaining a worse KS measure than was found for the observed energy distribution is 33% for the Kamiokande data and 65% for the IMB data.

Monte Carlo simulations show that (5% significance) between 3 and 7 of the first 8 events in Kamiokande were due to absorption of electron anti-neutrinos. The KS test can only reject the possibility of 0 scattering events at 2% significance, which is small but not completely negligible. There were between 6 and 8 absorption events in IMB (95% confidence level).

Figure 2a shows that there is a well-defined range of anti-neutrino fluxes,

$$F_{\bar{\nu}_e} = (0.15-0.7) \times 10^{10} \text{ cm}^{-2} \quad (3b)$$

(95% confidence level) that is consistent with both the observed event rates. If we require that the count rates in IMB and Kamiokande are compatible and assume that the detector efficiencies are accurately known^{1,2}, then the neutrino temperature must exceed 3.7 MeV.

For the temperature and flux ranges inferred here (equations (3a) and (3b)), we would not expect a large signal in either the Mont Blanc or the Baksan scintillator detectors⁸. Assuming a detection efficiency of 100%, we estimate $0.8 \times (T/4.1 \text{ MeV})^2 (F_{\bar{\nu}_e}/0.5 \times 10^{10} \text{ cm}^{-2})$ absorption events per 100 metric tons of scintillator.

As was already seen from Fig. 1, a much larger range is acceptable for the flux of ν_{scatt} , which is determined by a small number of events in the forward peak of either detector. The ν_{scatt} flux is harder to determine than the $\bar{\nu}_e$ flux because the scattering cross-section is much smaller than the absorption cross-section.

The strongest experimental limit on F_{scatt} comes from the detection of one to five (forward-peaked) scattering events in Kamiokande (see discussion of Monte Carlo experiments preceding Fig. 2a). The likelihood function does not vary significantly over this range but is peaked between two and three scattering events. Figure 2b shows that there is a large range of fluxes consistent at the 95% confidence level with one to five scattering events in Kamiokande. All fluxes in this range are consistent with the detection of 0 to 2 scattering events in IMB.

The formation of a neutron star in a type II supernova is expected to release, in neutrinos, nearly all of the star's binding energy, $0.1 \times M_{\text{neutron star}}$, which is 2.6×10^{53} ergs for a neutron star of mass $1.4 M_{\odot}$. Requiring that the energy emitted in neutrinos does not exceed the energy released by the collapse of the core to form a neutron star further constrains F_{scatt} . The total energy, E_{total} , that is removed by neutrinos is $E_{\text{total}} = 5.9 \times 10^{52} \text{ erg } (F/10^{10} \text{ cm}^{-2}) (T/4.1 \text{ MeV}) (D/50 \text{ kpc})^2$. The region above the dashed line in Fig. 2b corresponds to a total emitted neutrino energy in excess of 3×10^{53} ergs. We adopt as the acceptable range,

$$F_{\text{scatt}} = (0.1-5) \times 10^{10} \text{ cm}^{-2} \quad (3c)$$

The upper limit in equation (3c) could be increased slightly if we assumed that the supernova produced a neutron star more massive than $1.44 M_{\odot}$, which subsequently cooled and collapsed to form a black hole.

Can the expected flux of ν_e be detected in the ^{37}Cl experiment of Davis? The calculated number of events occurring in the ^{37}Cl tank, corresponding to the range of fluxes given in equation (3c), varies from 0.02 to 2, depending upon whether one uses the upper limit (with $F(\nu_e) = F_{\text{scatt}}$) or the lower limit (with $F(\nu_e) = 0.5 \times F_{\text{scatt}}$). The background from solar neutrinos will probably be too large to permit a stringent test of equation (3c).

The mass of $\bar{\nu}_e$ is strongly constrained⁹ by the observed energies and arrival times of the lowest-energy neutrino events if these events are due to absorption and not scattering (otherwise, the kinematic relations are not unique). The most important event has the lowest energy, 7.5 MeV, and is observed at an angle of $108^\circ \pm 32^\circ$ with respect to the LMC; the probability that this event is due to scattering is $< 0.2\%$. The two next most important events have measured energies $9.2 \text{ MeV } (70^\circ \pm 30^\circ)$ and $12.8 \text{ MeV } (135^\circ \pm 23^\circ)$; the corresponding probabilities that these events are due to scattering are, respectively, 5% and $< 0.1\%$. We conclude that it is valid to use absorption kinematics in constraining the mass of $\bar{\nu}_e$.

We now consider the following question: is there any evidence for or against matter oscillations of neutrinos (the MSW effect^{3,4}) having affected the neutrino fluxes or spectra? Yes, provided we consider the unfamiliar case in which the electron's neutrino is predominantly associated with the highest mass eigenstate. The ν_μ and ν_τ and their anti-neutrinos, are expected on conventional supernova models (see, for example, Table 1 of ref. 5) to be produced in comparable numbers to ν_e and $\bar{\nu}_e$, but with a temperature that may be a factor of two higher ($\sim 10 \text{ MeV}$). The key point is that the higher-temperature mu and tau anti-neutrinos can, for the unconventional mass hierarchy we stress here, be converted to more easily observable electron anti-neutrinos by neutrino oscillations.

If, on the other hand, the mixing angles are all small and the lowest mass eigenstate is almost pure ν_e , matter oscillations constitute an attractive solution³ of the solar neutrino problem, one that is consistent with some grand unified theories. However, as pointed out earlier in a prescient study², the expected effects on supernova neutrinos are not large for matter oscillations satisfying the above assumptions. Wolfenstein¹⁰ has noted that large mixing angles are also possible and may, for certain parameters, both solve the solar neutrino problem and have appreciable effects on the observed supernova neutrino spectra and fluxes.

What limits can be placed on possible high-temperature components of ν_{scatter} and $\bar{\nu}_e$? From the total number of events observed in the IMB detector, we find that the upper limit (95% confidence level) on the number of high temperature, $T > 10$ MeV, neutrinos is

$$F_{\bar{\nu}_e}(T > 10 \text{ MeV}) \leq 0.1 \times 10^{10} \text{ cm}^{-2} (10 \text{ MeV}/T) \quad (4a)$$

and, from the angular distribution of the IMB events and the total energy constraint (see discussion preceding equation (3c))

$$F_{\text{scatt}}(T > 10 \text{ MeV}) \leq 2 \times 10^{10} \text{ cm}^{-2} (10 \text{ MeV}/T) \quad (4b)$$

But if matter oscillations with large mixing angles do occur and $\bar{\nu}_e$, $\bar{\nu}_\mu$ and $\bar{\nu}_\tau$ were produced in comparable numbers, then one might expect to have observed a high-temperature component of $\bar{\nu}_e$ that represented converted mu and tau anti-neutrinos. As both $\bar{\nu}_\mu$ and $\bar{\nu}_\tau$ could have been converted to $\bar{\nu}_e$, favourable conditions would have resulted in a flux of about twice as many (converted) high temperature $\bar{\nu}_e$ as direct low-temperature $\bar{\nu}_e$. Equation (4a) shows that this did not occur. In fact, this conceivable manifestation of the MSW effect did not occur at a level of 0.1 or more above what might have been expected.

Our main conclusions are summarized below. (1) The temperatures and fluxes (equations (3a-c)) inferred here are in remarkable agreement with the standard theoretical ideas of how stars collapse and supernovae are formed¹¹⁻²¹; the observations of supernova neutrinos represent therefore a great triumph for astrophysical theory. Table 1 of ref. 5 gives a convenient summary of the (pre-observation) expectations. The numerical comparison between observations (equation (3)) and expectations (Table 1 of ref. 5) is: $T_{\text{expect}} = 5 \text{ MeV}$ [$T_{\text{obs}} = 4.1^{+1.0}_{-0.4} \text{ MeV}$]; $F_{\text{expect}} = 1.1 \times 10^{10} \text{ cm}^{-2}$; [$F_{\text{obs}} = 0.5^{+0.2}_{-0.35} \times 10^{10} \text{ cm}^{-2}$]; and $F_{\text{expect}}(\nu_e) = 1.6 \times 10^{10} \text{ cm}^{-2}$ [$F_{\text{obs}}(\text{scatt}) = (0.1-5) \times 10^{10} \text{ cm}^{-2}$]. The total energy in neutrinos is, within the uncertainties, consistent with the theoretical expectation of 3×10^{53} ergs. This model suggests that the full (3 kton) Kamiokande detector experienced $\sim 20(T/4.1 \text{ MeV})^2 (\text{Flux}/0.5 \times 10^{10} \text{ cm}^{-2})$ absorption events (assuming 100% efficiency). (2) The simplest (one-temperature thermal) model provides an acceptable fit to the observed energy and angular distributions of both the Kamiokande and the IMB observations. The success of the simple model means that major refinements are not required by the data, except perhaps to explain the three delayed events observed in the Kamiokande detector⁶. One can use the observations to rule out more detailed and specific astrophysical models, but it seems unlikely that one will be able to infer many additional parameters in an unambiguous fashion. (3) Between 3 and 7 absorption events were observed in Kamiokande and between 6 and 8 absorption events were detected by IMB (5% significance). The possibility of zero scattering events in both detectors can only be rejected at the level of 2% significance. The crucial low-energy events, which are important for setting an upper limit on the mass of $\bar{\nu}_e$, can safely be ascribed to absorption, not scattering. (4) We expect⁵ only a small number of events in the Mont Blanc, Baksan and Homestake (³⁷Cl) detectors. (5) The observations constrain possible high-temperature neutrino fluxes (see equation (4)), and argue against the occurrence of the MSW effect in an unconventional mass hierarchy in which electron neutrinos are predominantly associated with the heaviest mass eigenstates.

While this work was being completed, we received several interesting and relevant papers (J. Arafune and S. Fukujita, preprint RIFP-693, Kyoto University, 1987; K. Sato and H. Suzuki, UTAP-47, University of Tokyo, 1987; refs 22, 23). Our paper is the first joint statistical analysis of the result in both the Kamiokande and the IMB experiments, which accounts for the fact that some of our conclusions are different from those given in the recent preprints.

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Note added in proof: J. C. van der Velde (personal communication) suggests that the best estimate for the effective volume of IMB may be 6.5 ktons. This larger amount of material improves slightly the joint fits, reduces the ridge-line for the temperature by 0.2 MeV (to 3.9 MeV), and does not significantly affect the estimated fluxes.

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- Hirata, K. *et al. Phys. Rev. Lett.* **58**, 1490-1493 (1987).
- Bionata, R. M. *et al. Phys. Rev. Lett.* **58**, 1494-1496 (1987).
- Mikheev, S. P. & Smirnov, A. Yu. *Nuovo Cimento* **9C**, 17-26 (1986).
- Wolfenstein, L. *Phys. Rev. D* **17**, 2369-2374 (1978).
- Bahcall, J., Dar, A. & Piran, A. *Nature* **326**, 135-136 (1987).
- Spergel, D. N., Piran, T., Loeb, A., Goodman, J. & Bahcall, J. N. *Phys. Rev. Lett.* (submitted).
- Bahcall, J. *Rev. mod. Phys.* **59**, (in the press).
- Badino, G. *et al. Nuovo Cim.* **7C**, 573-590 (1984).
- Bahcall, J. N. & Glashow, S. L. *Nature* **326**, 476-477 (1987).
- Walker, T. P. & Schramm, D. N. *Phys. Lett.* (in the press).
- Baade, W. & Zwicky, F. *Proc. natn. Acad. Sci.* **20**, 254-259 (1934).
- Fowler, W. A. & Hoyle, F. *Astrophys. J. Suppl. Ser.* **9**, 201-319 (1964).
- Colgate, S. A. & White, R. H. *Astrophys. J.* **143**, 626-681 (1966).
- Arnett, W. D. *A. Rev. Astr. Astrophys.* **11**, 73-94 (1973).
- Bethe, H. A. *et al. Nucl. Phys.* **A324**, 487-533 (1979).
- Bowers, R. L. & Wilson, J. R. *Astr. J. Suppl. Ser.* **50**, 115-160 (1982).
- Arnett, W. D. *Astrophys. J.* **263**, L55-L57 (1983).
- Bethe, H. A. & Wilson, J. R. *Astrophys. J.* **295**, 14-23 (1985).
- Bethe, H. A. & Brown, G. *Scient. Am.* **252**, 60-68 (1985).
- Burrows, A. & Lattimer, J. M. *Astrophys. J.* **307**, 178-196 (1985).
- Wilson, J. R., Mayle, R., Woosley, S. E. & Weaver, T. *Ann. N. Y. Acad. Sci.* **470**, 267-293 (1986).
- Hayano, R. S. & Ishikawa, T. *Phys. Rev. Lett.* (submitted).
- Hillebrand, W. *et al. Astr. Astrophys.* (submitted).

Downflows in coronal loops

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The existence of the solar wind requires a net outflow of material at all depths in the solar atmosphere. Thus, the observation of a prevalent downflow at temperatures near $10^5 \text{ K}^{1,2}$ presents a considerable challenge. Two classes of flow models have been suggested, each involving flow along closed magnetic-field lines that extend above the chromosphere into the hotter transition region and corona. Type-(1) models^{3,4,5} assume unidirectional equilibrium flows along field lines and rely on asymmetry in the flow and plasma properties to produce an apparent downflow. Type-(2) models^{2,6}, by comparison, assume a series of episodic upflows and subsequent downflows along each line of force and rely on asymmetry in the plasma properties and flow durations to produce a statistically prevalent downflow. Here I argue that observational evidence strongly suggests that both types of flow are present but that type-(2) flow is predominant.

To establish this conclusion, we first review the observed properties of commonly occurring flows. Three features of the flow that have been extensively documented are as follows. (1) The apparent downflow velocity^{1,2} reaches a maximum near 10^5 K and is markedly diminished near $2 \times 10^5 \text{ K}$. (2) An upflow in the form of cool spicules^{7,8} is observed near $1.5 \times 10^4 \text{ K}$ and has a mass flux^{6,9} that agrees well with the reverse mass flux observed near 10^5 K . (3) Large-scale patterns of alternate bands of blue- and redshift separated by velocity neutral lines^{10,11,12} observed near 10^5 K are well correlated with large-scale magnetic field patterns at the photospheric level, but for each magnetic neutral line there are typically two velocity neutral lines. For convenience, we refer to the former as B_0 lines and the latter as V_0 lines. In the following, we draw particular attention to the tendency for two V_0 lines to accompany each B_0 line. This observation holds an important clue to the true nature of the flow field.

It is important to note that both the velocity and magnetic maps give only the line-of-sight component of the field. Thus,

a neutral line occurs whenever the field vector is either zero or is oriented perpendicular to the line of sight. It seems clear that neutral lines in the large-scale magnetic field are nearly always of the latter type and that the B_0 lines most commonly lie along the axes of magnetic arcades. Thus, we expect a one-to-one association between large-scale B_0 lines and arcades of magnetic loops. Such a picture has long been advocated in connection with coronal helmet streamers and quiescent prominences.

If there is, indeed, a set of magnetic loops arching over most magnetic neutral lines in the large-scale field, it follows from (3) that each arcade of loops typically exhibits two V_0 lines. The only possibility for this pattern of two V_0 lines in each arcade to be a consistent feature of the flow field is for one of the V_0 lines to be a line of zero velocity and the other to be a line on which the velocity vector is normal to the line of sight. The most usual circumstance in an arcade viewed at a particular angle will result in a single locus of points along which the field lines are perpendicular to the line of sight. Thus, in any pattern of flow along field lines, there will normally be no more than one V_0 line of this type. It follows that the remaining V_0 line must represent a line along which the velocity vector has zero amplitude.

The most plausible place for zero velocity is at the top of the loops, which suggests a model with downflow in both legs. When the arcade is viewed in a particular spectral line, the observed velocity signal is representative, more or less, of the flow in a slab cutting horizontally through the arcade, as illustrated in Fig. 1. When viewed away from disk centre, such a cut will show two V_0 lines, one where the shell is tangent to the tops of the inner loops, and a second where the shell cuts across outer loops that are locally perpendicular to the line of sight. If the flow were unidirectional throughout the arcade, only the latter V_0 line would be observed. It is important to note that the sets of loops with which the two V_0 lines are observed are very different in character. The former of the two V_0 lines, when observed at 10^5 K, is at the crests of low-lying loops in the core of the arcade, whereas the latter lies in the legs of loops that may extend well into the corona.

A representation of the type just described has been shown¹² to account satisfactorily for most of the detailed correlation¹¹ between the velocity patterns observed in C IV lines and photospheric magnetic fields. The observations on which this representation is made relate primarily to the regions of intermediate field strength in and near active regions. In quiet Sun regions both B_0 and V_0 lines occur, but there are insufficient observations to establish the detailed correlation between them. In the strong fields of plages and sunspots the flow is consistently downwards¹¹. The latter, of course, is consistent with either type-(1) or type-(2) flows.

Until now, there have been no quantitative models of the type represented in Fig. 1 owing to the relative complexity of the model. Instead, published models have concentrated on unidirectional flow between opposite foot points of a magnetic loop. The model of Cargill and Priest⁵ is representative of a class of models in which the flow is driven by pressure differences imposed as boundary conditions at the foot points. One then uses an asymmetric loop geometry in an attempt to show a predominance of downflow in the observations at a given temperature. A second class of models by Mariska and Boris⁴ and more recently McClymont and Craig³ rely on asymmetric heating within the loop to create pressure-driven flows. The latter model, in particular, successfully reproduces an apparent downflow of the proper magnitude. However, none of the models relying on unidirectional flow in magnetic loops satisfactorily accounts for the existence of prominent V_0 lines.

The apparent nearly universal tendency for downflow at 10^5 K can be explained by type-(1) flow only if the autocorrelation length for the flow direction is very short when measured perpendicular to field lines. In other words, there cannot be large areas in which the flow direction is consistently in the same sense

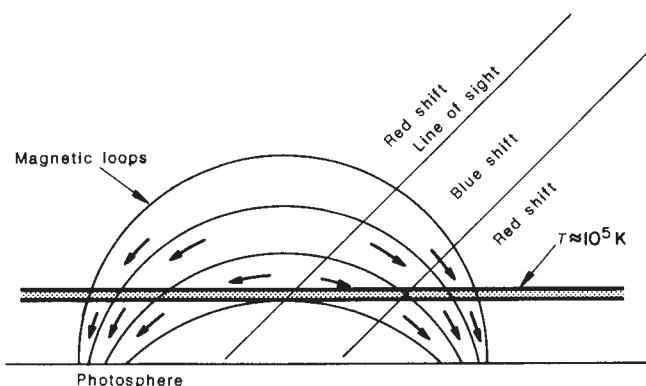


Fig. 1 Formation of two V_0 lines in a magnetic arcade with downflow in both sides of the arcade. The view is along the axis of the arcade.

with respect to the magnetic polarity. Otherwise, there would be large areas of the solar disk with observed upflow. It follows that for a given arcade of loops with type-(1) flow, the locus of points along which the field lines are perpendicular to the line of sight would show a V_0 line along which the flow direction showed frequent changes in sign over short distances. The observed V_0 lines, however, are consistently of one polarity over large distances.

When viewed away from disk centre, the type-(2) flow pattern illustrated in Fig. 1 will typically show two V_0 lines separating an inner zone of blueshift from two bordering zones of redshift. This pattern is consistent, in all respects, with the observed properties of the flows bordering active regions^{11,12}. Thus, the type-(2) flow fits quite naturally with the observed properties of V_0 lines, whereas the type-(1) flow leads to direct conflict with the same observation. Still other evidence in favour of type-(2) flow comes from a different aspect of the velocity data.

In studies of individual loops¹³, observed in C IV lines formed at temperatures near 10^5 K, there are many examples in which the flow is unidirectional from one end of the loop to the other. Thus, flow patterns of type (1) as proposed recently by McClymont and Craig³ clearly have an element of realism. On the other hand, this same study of individual loops¹³ shows that most often there is an apparent simultaneous downflow at both footpoints of the loop¹³. Thus, the data support the existence of type-(1) flow but again favour type-(2) flow as being the most common.

Because models of type (1) showing ubiquitous downflow are not consistent with observations of V_0 lines, we seem forced to deal with models of type (2). The illustration in Fig. 1 is incomplete, of course, because there is no indication of upflow. The most logical way of including an upflow is to assume that both the upflow and downflow occur in the same flux tubes^{2,6}, which implies that the flows are episodic (upflow followed by downflow). It is noteworthy, therefore, that the upward mass flux estimated for spicules agrees closely with the estimated downward mass flux near 10^5 K^{6,9}. Because spicules are known to be at temperatures much below 10^5 K⁸, the suggested type-(2) flow is completed by assuming that the cool spicule material is heated to temperatures near 10^5 K before it descends. Such a model has the benefit of satisfying all aspects of the observations in a qualitative sense, but no quantitative models exist. Before such modelling can be performed in a convincing manner, it will be necessary to understand both the acceleration and heating of spicule material, neither of which we have achieved.

A consequence of identifying spicules as the dominant source of upflow is that steady heating becomes an unlikely candidate as the driving force. Pikel'ner¹⁴ has noted that in a pressure-driven vertical flow, the pressure gradient must exceed the hydrostatic gradient, whereas in spicules the reverse is true⁸. In

fact, the axial pressure gradient in the rising spicule material is only a small fraction of the hydrostatic gradient defined by the spicule temperature. Although this may not eliminate impulsive heating as a possible driving mechanism, it provides a strong argument against steady heating.

A complicating feature of the type-(2) flow is that it is impulsive and statistical rather than steady state. On the other hand, the transition region near 10^5 K is noted for the short-lived, dynamic character¹⁵ of the small-scale features that dominate its structure. It should come as no surprise, therefore, that steady-state models of the flow prove to be oversimplified.

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1. Doschek, G. A., Feldman, U. & Bohlén, J. D. *Astrophys. J.* **205**, L177-L180 (1976).
2. Feldmann, U., Cohen, L. & Doschek, G. A. *Astrophys. J.* **255**, 325-328 (1982).
3. McClymont, A. N. & Craig, I. J. D. *Nature* **324**, 128-129 (1986).
4. Mariska, J. T. & Boris, J. P. *Astrophys. J.* **267**, 409-420 (1983).
5. Cargill, P. J. & Priest, E. R. *Sol. Phys.* **65**, 251-269 (1980).
6. Athay, R. G. & Holzer, T. E. *Astrophys. J.* **255**, 743-752 (1982).
7. Thomas, R. N. & Athay, R. G. *Physics of the Solar Chromosphere* (Interscience, New York, 1961).
8. Beckers, J. W. A. *Rev. Astr. Astrophys.* **10**, 73-100 (1972).
9. Pneuman, G. W. & Kopp, R. A. *Sol. Phys.* **57**, 49-64 (1978).
10. Athay, R. G., Gurman, J. B., Henze, W. & Shine, R. A. *Astrophys. J.* **261**, 684-699 (1982).
11. Klimchuk, J. A. thesis, Univ. (1985).
12. Athay, R. G. & Klimchuk, J. A. *Astrophys. J.* (in the press).
13. Athay, R. G., Gurman, J. B. & Henze, W. *Astrophys. J.* **269**, 706-714 (1983).
14. Pikel'ner, S. B. *Astrophys. Space Phys.* **3**, 33-38 (1971).
15. Dere, K. P., Bartoe, J.-D. F. & Brueckner, G. E. *Astrophys. J.* **281**, 870-883 (1984).

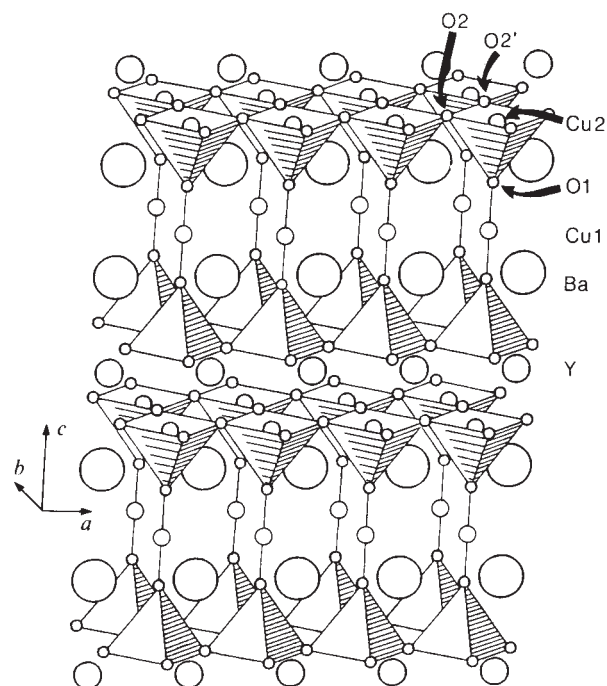


Fig. 1 Structure of $\text{YBa}_2\text{Cu}_3\text{O}_6$, showing the CuO_5 pyramids (horizontally hatched) and CuO_2 linear bonds. The barium and yttrium cations are drawn to scale. O_2 and O_2' are equivalent in $\text{YBa}_2\text{Cu}_3\text{O}_6$; in the orthorhombic $\text{YBa}_2\text{Cu}_3\text{O}_7$, O_2' is O_3 .

Crystal structure of $\text{Y}_{0.9}\text{Ba}_{2.1}\text{Cu}_3\text{O}_6$, a compound related to the high- T_c superconductor $\text{YBa}_2\text{Cu}_3\text{O}_7$

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The crystal structure of the high-temperature superconductor $\text{YBa}_2\text{Cu}_3\text{O}_7$ is derived from the ideal perovskite ($\text{YBa}_2\text{Cu}_3\text{O}_9$) structure by the presence of ordered vacancies in the oxygen sublattice. Owing to the ordering of these vacancies, the coordination polyhedra around the copper cations are either pyramids or slightly distorted squares¹⁻⁵; the one-dimensional character and the orthorhombic symmetry of the structure are due to the existence of the squares. We have synthesized single crystals of the further-reduced compound $\text{Y}_{0.9}\text{Ba}_{2.1}\text{Cu}_3\text{O}_6$, and we report here a structural determination using single-crystal X-ray diffraction data. $\text{Y}_{0.9}\text{Ba}_{2.1}\text{Cu}_3\text{O}_6$ has a tetragonal oxygen-deficient perovskite structure related to that of $\text{YBa}_2\text{Cu}_3\text{O}_7$. The additional oxygen vacancies transform the CuO_4 squares into O—Cu—O linear bonds, while the pyramids are not affected. As $\text{Y}_{0.9}\text{Ba}_{2.1}\text{Cu}_3\text{O}_6$ does not exhibit any superconducting transition down to 4.2 K, we conclude that the chains of CuO_4 squares are essential for the superconductivity mechanism.

When heated to $>500^\circ\text{C}$, the $\text{YBa}_2\text{Cu}_3\text{O}_7$ compound can reversibly lose oxygen; a composition close to $\text{YBa}_2\text{Cu}_3\text{O}_6$ can be obtained by heat treatment at 920°C and 10^{-2} atm oxygen partial pressure. This oxygen loss is associated with a phase transformation in which the symmetry changes from orthorhombic to tetragonal. This transformation occurs between 600 and 650°C in air and is reversible⁶. The amount of oxygen lost is entirely reabsorbed on cooling, provided the cooling rate is not too high and the sample is in the presence of enough oxygen.

Plate-like single crystals of $\text{YBa}_2\text{Cu}_3\text{O}_{7-x}$, $\sim 50\ \mu\text{m}$ thick and $\sim 200\ \mu\text{m}$ in diameter, grow naturally when a mixture containing 4BaCO_3 , Y_2O_3 and 6CuO is heated at 950°C for 12 hours and quenched in air. It seems that the growth is due to the existence of a CuO-rich liquid phase which forms between 900 and 950°C during sintering. To prepare larger single crystals, we tried to increase the quantity of this liquid phase by adding a 20% excess of CuO and following the same synthesis procedure. The X-ray powder analysis revealed that these samples contained more than one phase. The single crystals proved to be tetragonal, with cell parameters $a = 3.8715(6)\ \text{\AA}$ and $c = 11.738(2)\ \text{\AA}$. These values are quite similar to those of orthorhombic $\text{YBa}_2\text{Cu}_3\text{O}_7$ ($a = 3.8206(1)\ \text{\AA}$, $b = 3.8851(1)\ \text{\AA}$, $c = 11.6757(4)\ \text{\AA}$). Susceptibility measurements showed no evidence of a superconducting transition down to 4.2 K. To elucidate the relationships between this non-superconducting phase and the high- T_c superconductor $\text{YBa}_2\text{Cu}_3\text{O}_7$, we have determined the formula and the crystal structure of this new tetragonal compound from single-crystal X-ray data.

A disk-shaped crystal, 0.026 mm thick and 0.26 mm in diameter, was selected. A preliminary study on a precession camera with Mo $K\alpha$ radiation confirmed that the crystal had tetragonal symmetry. No systematic extinctions were found among the reflections. The disk was then mounted on a Nonius CAD4 diffractometer equipped with graphite-monochromated Ag $K\alpha$ radiation. All reflections belonging to the shell of reciprocal space between $\theta = 2^\circ$ and $\theta = 34^\circ$ were collected. Corrections for the Lorentz and polarization effects were applied, along with an empirical absorption correction following the method described in ref. 7.

The Patterson map was calculated with the 477 averaged reflections; the most intense peaks corresponded to the Ba—Y, Ba—Cu1, Ba—Cu2, Y—Cu1 and Y—Cu2 inter-atomic vectors found in $\text{YBa}_2\text{Cu}_3\text{O}_7$ (space group $Pmmm$). The refinements were started with these atoms placed in the corresponding positions of the $P4/mmm$ space group. Difference Fourier maps revealed the positions of the oxygen atoms: O1 at $(0, 0, z)$, $z = 0.16$ and O2 at $(\frac{1}{2}, 0, z)$, $z = 0.38$, corresponding to the atoms O1 and

Table 1 Positional, thermal and occupancy parameters for $\text{YBa}_2\text{Cu}_3\text{O}_6$

	Wyckoff symbol	x	y	z	U_{11}	U_{22}	U_{33}
Ba	2h	1/2	1/2	0.1921(1)	0.0105(2)	$= U_{11}$	0.0148(4)
Y*	1d	1/2	1/2	1/2	0.0051(5)	$= U_{11}$	0.0111(9)
Cu1	1a	0	0	0	0.0193(8)	$= U_{11}$	0.017(2)
Cu2	2g	0	0	0.3605(2)	0.0029(3)	$= U_{11}$	0.0129(8)
O1	2g	0	0	0.154(1)	0.018(2)	$= U_{11}$	0.010(5)
O2	4l	1/2	0	0.3794(7)	0.003(2)	0.009(2)	0.015(3)

* $\text{Y} = 0.89(4)\text{Y} + 0.11(4)\text{Ba}$

(O2, O3) of $\text{YBa}_2\text{Cu}_3\text{O}_7$, respectively. The refinements were carried out by the Wolfers MXD program with 309 reflections having intensity $I_{\text{obs}} > 5\sigma(I_{\text{obs}})$ and $\sin\theta/\lambda > 0.36$. All atoms had anisotropic thermal parameters, and the occupancy factors of the Y, O1 and O2 atoms were also varied. No significant change with refinement was observed for the O1 and O2 occupancy factors, whereas that of the Y atom increased to a value of 1.05(1) and the R and weighted R factors improved by 10%. Moreover, if the Y occupancy factor was kept fixed at 1.00, a residual peak was present in the Fourier difference map. We therefore allowed a substitution of barium on the yttrium site. In the last cycles of refinement, all positional and thermal parameters were varied along with the Y/Ba ratio on the yttrium site, yielding the agreement factors $R = 4.6\%$ and weighted $R = 3.3\%$. The composition of the yttrium site was found to be $0.89(4)\text{Y} + 0.11(4)\text{Ba}$. Attempts to put an oxygen atom at the position corresponding to the O4 atoms and O5 vacancies of $\text{YBa}_2\text{Cu}_3\text{O}_7$ led to a zero occupancy factor, so these atoms were removed. Beech *et al.*³ showed by powder neutron diffraction that the additional oxygen vacancies in a compound such as $\text{YBa}_2\text{Cu}_3\text{O}_{6.8}$ occurred on the O4 position of the $\text{YBa}_2\text{Cu}_3\text{O}_7$ structure. This means that on going from $\text{YBa}_2\text{Cu}_3\text{O}_7$ to $\text{YBa}_2\text{Cu}_3\text{O}_6$ the O4 sites are gradually emptied. Although the correct stoichiometry for the crystal studied is $\text{Y}_{0.9}\text{Ba}_{2.1}\text{Cu}_3\text{O}_6$, we will refer to it below as $\text{YBa}_2\text{Cu}_3\text{O}_6$.

The positional, thermal and occupancy parameters are given in Table 1, the principal interatomic distances in Table 2. The cell parameters were determined from a Guinier powder film taken with Fe $K\alpha$ radiation and Si standard. The least-squares refinement yielded $a = 3.8715(6)$ Å, $c = 11.738(2)$ Å.

As shown in Fig. 1, the structure of $\text{YBa}_2\text{Cu}_3\text{O}_6$ can still be considered as an oxygen-deficient perovskite, although in this compound the cation to anion ratio has increased to 1 from 2/3 for the ABO_3 stoichiometric perovskite compound. As in $\text{YBa}_2\text{Cu}_3\text{O}_7$, the Ba and Y cations are ordered so as to form layers parallel to the a - b plane, in the sequence Ba-Y-Ba-Ba-Y.

The $\text{YBa}_2\text{Cu}_3\text{O}_6$ structure is obtained from $\text{YBa}_2\text{Cu}_3\text{O}_7$ by removing the O4 atoms. This changes the square coordination of the Cu1 cations to a linear O-Cu1-O coordination. As the a and b directions become equivalent, the symmetry changes from orthorhombic to tetragonal.

In $\text{YBa}_2\text{Cu}_3\text{O}_6$, the Ba cations are surrounded by eight oxygen atoms arranged as an antiprism. One square has edges equal to the a parameter; the other has edges equal to $a/\sqrt{2}$. The average Ba-O distance is 2.852 Å, as compared with 2.860 Å for the corresponding distance in $\text{YBa}_2\text{Cu}_3\text{O}_7$. This near equality is surprising because in the latter compound the Ba cations are surrounded by ten oxygen atoms, and a somewhat larger Ba-O distance would be expected.

The Y-O average distance has the same value in $\text{YBa}_2\text{Cu}_3\text{O}_7$ and in $\text{YBa}_2\text{Cu}_3\text{O}_6$. This too is surprising, because although the Y sites have the same coordination in both compounds, in the latter the Y sites are occupied by 89% Y and 11% Ba. Such an occupation factor should correspond to a 2% larger cation-oxygen average distance.

The Cu1 cation sites in $\text{YBa}_2\text{Cu}_3\text{O}_6$ are surrounded by two oxygen atoms; this coordination is typical for Cu^{1+} cations. In Cu_2O , in which the copper cations have this type of coordination,

the Cu-O distance is 1.85 Å, much larger than that found in $\text{YBa}_2\text{Cu}_3\text{O}_6$ (1.80 Å).

The Cu2 cation sites in $\text{YBa}_2\text{Cu}_3\text{O}_6$ are surrounded by five oxygen atoms arranged as an apically elongated square pyramid. The four oxygen atoms forming the square are at 1.948 Å—about the same as the corresponding Cu2-O distance in $\text{YBa}_2\text{Cu}_3\text{O}_7$. But the pyramid elongation is quite different: the Cu2-O1 distance is 2.43(1) Å in $\text{YBa}_2\text{Cu}_3\text{O}_6$, as compared with 2.30(1) Å in $\text{YBa}_2\text{Cu}_3\text{O}_7$. The larger distance is due to the fact that in the former compound the Cu2 sites are occupied by Cu^{2+} cations, whereas an average valence of 2.25+ was found for the Cu2 sites in $\text{YBa}_2\text{Cu}_3\text{O}_7$.

Qualitatively, the interatomic distances seem to indicate that in $\text{YBa}_2\text{Cu}_3\text{O}_6$ the Cu^{1+} and Cu^{2+} cations are localized on the Cu1 and Cu2 sites, respectively. As stated above, the Cu1-O1 distance is much smaller than expected. As can be seen in Fig. 1, the O1-Cu1-O1 bonds, which link together the pyramidal layers, are essential for the stability of the structure. In order for the structure to exist these bonds must be very strong, and consequently the corresponding Cu1-O1 distances must be as small as possible. The shortness of the Cu1-O1 bonds is responsible for the large elongation of the pyramid around the Cu2 cations.

The barium excess is compensated either by additional oxygen vacancies or by an increase of the copper valence. It is quite unlikely that the additional vacancies would occur on the O1

Table 2 Interatomic distances for $\text{Y}_{0.9}\text{Ba}_{2.1}\text{Cu}_3\text{O}_6$ and $\text{YBa}_2\text{Cu}_3\text{O}_7$

Bond	$\text{Y}_{0.9}\text{Ba}_{2.1}\text{Cu}_3\text{O}_6$		$\text{YBa}_2\text{Cu}_3\text{O}_7$	
	Distance (Å)	Number	Distance (Å)	Number
Cation-oxygen distances				
Ba-O1*	2.775(3)	×4	Ba-O1	2.741(1) ×4
Ba-O2	2.929(7)	×4	Ba-O2	2.982(5) ×2
Average	2.852		Ba-O3	2.959(6) ×2
			Ba-O4	2.876(4) ×2
			Average	2.860
Y-O2	2.399(6)	×8	Y-O2	2.409(3) ×4
			Y-O3	2.385(3) ×4
			Average	2.397
Cu1-O1	1.804(16)		Cu1-O1	1.846(5) ×2
			Cu1-O4	1.942(0) ×2
			Average	1.894
Cu2-O1	2.428(16)	×1	Cu2-O1	2.298(6) ×1
Cu2-O2	1.948(1)	×4	Cu2-O2	1.929(1) ×2
Average	2.044		Cu2-O3	1.961(1) ×2
			Average	2.015
Metal-metal distances				
Ba-Ba	3.8715(6)		Ba-Ba	3.8851(1)
Ba-Y	3.614(1)		Ba-Y	3.688(5)
Ba-Cu1	3.5468(7)		Ba-Cu1	3.470(3)
Ba-Cu2	3.377(1)		Ba-Cu2	3.376(3)
Y-Cu2	3.190(1)		Y-Cu2	3.208(2)
Cu1-Cu2	4.232(2)		Cu1-Cu2	4.144(4)
Cu2-Cu2	3.274(1)		Cu2-Cu2	3.388(2)
Oxygen-oxygen distances				
O1-O2	3.281(15)		O1-O2	3.199(5)
O2-O2	2.738(6)		O1-O3	3.217(6)
			O2-O3	2.7245(0)

* Oxygen nomenclature differs from refs 1 and 4 (O1 and O4 interchanged).

sites (see Fig. 1). The refinement of the O2 occupancy parameter did not yield any conclusive result. If all the barium excess were compensated by O2 vacancies, the departure from full occupancy would be of the same order of magnitude as the standard deviation of the occupancy parameter. The Cu valence could increase either on the Cu1 sites from 1+ to 2+, or on the Cu2 sites from 2+ to 3+. It must be pointed out that linear twofold coordination for Cu²⁺ cations is unknown, while the second mechanism implies that a Cu¹⁺ cation would have a Cu³⁺ as second-nearest-neighbour. We do not have any experimental evidence for choosing one or the other.

It has been shown (J. J. Capponi *et al.*, unpublished data, and ref. 8) that the oxygen loss from YBa₂Cu₃O₇ to YBa₂Cu₃O_{7-x}, with the Ba/Y ratio equal to 2:1, is reversible when the sample is heat-treated at the right temperature and under the proper atmosphere. As shown by thermogravimetry, the crystals of Y_{0.9}Ba_{2.1}Cu₃O₆ do not gain any oxygen when heat-treated under the same conditions. Powder photographs taken before and after the heat treatment show that no transformation has taken place. They can be indexed on the same tetragonal cell. Why the barium excess prevents the oxidation to YBa₂Cu₃O₇ is not clear at this point.

The YBa₂Cu₃O₆ compound has not been found to become superconducting down to 4.2 K (J. L. Tholence, personal communication). If the deduction of Cu¹⁺ on Cu1 and Cu²⁺ on Cu2 is correct, then the implication is that the 3d bands on the Cu1 sites are full, and as long as the 4s bands do not overlap, then one expects the Cu1 sites to be insulating. Since there is no large change to the Cu2 sites, one may deduce that the chains of CuO₄ squares are important for the superconducting properties. We cannot say whether the presence of Cu³⁺ cations is essential for the existence of superconductivity.

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1. Beno, M. A. *et al.* *Appl. Phys. Lett.* (in the press).
2. Capponi, J. J. *et al.* *Europhys. Lett.* **3**, 1301-1308 (1987).
3. Beech, F., Miraglia, S., Santoro, A. & Roth, R. S. *Phys. Rev. Lett.* (in the press).
4. David, W. I. F. *et al.* *Nature* **327**, 310-312 (1987).
5. Gredan, J. E., O'Reilly, A. & Stager, C. V. *Phys. Rev.* (submitted).
6. Strobel, P., Capponi, J. J., Chailout, C., Marezio, M. & Tholence, J. L. *Nature* **327**, 306-308 (1987).
7. North, A. C. T., Phillips, D. C., Mathews, F. S. *Acta crystallogr.* **A24**, 351 (1968).
8. Schuller, I. K. *et al.* *Solid St. Commun.* (in the press).

Large heterogeneous ²⁶Mg excesses in a hibonite from the Murchison meteorite

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The correlation of excess ²⁶Mg with Al/Mg ratio in coexisting phases of refractory inclusions from carbonaceous chondrites has been cited as evidence for the *in situ* decay of ²⁶Al in the early Solar System¹⁻⁸; many refractory inclusions indicate an initial ²⁶Al/²⁷Al of $\sim 5 \times 10^{-5}$. On the other hand, the short half life of ²⁶Al, and its apparently high activity in the interstellar medium as measured by γ -ray spectroscopy⁹, suggest that interstellar dust could be enriched in ²⁶Mg^{10,11}. In this model, the ²⁶Mg excesses measured in refractory inclusions are the result of 'chemical memory' of the constituent material. Here we report measurements of ²⁶Mg excesses approaching 400% in a hibonite, grain 7-971, from the Murchison carbonaceous chondrite. The excesses are not homogeneous: the core is enriched by $355 \pm 6\%$, at the 95% confidence limit, whereas an analysis nearer the edge has a lower excess of $145 \pm 6\%$. The magnesium in 7-971 is also significantly mass-fractionated, with the heavy isotopes enriched relative to terrestrial compositions, and this is a possible signature of a distillation event. The magnesium isotope systematics of this hibonite are compatible with models incorporating either *in situ* ²⁶Al decay and/or chemical memory of exotic Mg, but the Al-Mg system was probably disturbed in a later thermal event.

Enrichments of ²⁶Mg from ²⁶Al decay are best observed in mineral phases having high Al/Mg ratios. Analyses of meteoritic hibonites (CaAl₁₂O₁₉) have been rather enigmatic in that despite their high Al/Mg ratios, and their probable early formation in the solar nebula, they have shown a wide range in the inferred ²⁶Al/²⁷Al from zero up to $\sim 10^{-3}$, with the majority being less than 10^{-5} (refs 4, 12-24). These huge variations question the interpretation that excess ²⁶Mg is due to a homogeneous distribution of ²⁶Al in the early solar nebula.

Meteoritic hibonite usually contains significant MgO (>0.5%), but a few hibonites with very low magnesium content have also been described. Hinton and Bischoff²¹ repor-

ted a hibonite from the ordinary chondrite Dhajala which had ²⁷Al/²⁴Mg ratios up to 1.7×10^4 , and with a correlated excess in ²⁶Mg of up to 100%. Despite the large excess ²⁶Mg, the inferred ²⁶Al/²⁷Al was only $8.4(\pm 0.5) \times 10^{-6}$. Lee *et al.*¹⁴ and Hinton and Bischoff²¹ did not find any excess ²⁶Mg in HAL hibonite, however Fahey *et al.*²² measured small excesses of ²⁶Mg, at ²⁷Al/²⁴Mg ratios of up to 4×10^4 , corresponding to an initial ²⁶Al/²⁷Al of $5.2(\pm 1.7) \times 10^{-8}$. Both of these hibonites showed large variations in ²⁷Al/²⁴Mg during the analyses, and the data defined ²⁶Mg/²⁴Mg intercepts which were, within error, identical to the terrestrial magnesium isotopic composition.

Another hibonite, 7-971, which has extremely low substitution of Ti and Mg (<100 p.p.m.) has been located in a mineral separate from the Murchison carbonaceous chondrite. The grain is an optically-uniform clear crystal approximately 100 μ m in diameter. Magnesium isotope analyses of 7-971 have been carried out on the ion microprobe SHRIMP with the procedures described by Ireland *et al.*²³, but using the ion counter to measure the low Mg⁺ isotope intensities rather than the Faraday cup.

The isotope ratios ²⁵Mg/²⁴Mg and ²⁶Mg/²⁴Mg were determined for each scan through the masses with 10-second count times at each peak. The fractionation, *F*, and residual, $\delta^{26}\text{Mg}$, were determined from the ratios, using

$$F = [(^{25}\text{Mg}/^{24}\text{Mg})/0.12663 - 1] \times 1,000$$

$$\delta^{26}\text{Mg} = [(^{26}\text{Mg}/^{24}\text{Mg})/0.139432 - 1] \times 1,000 - 2F$$

The fractionation is the relative deviation, expressed in per mil per AMU, from the terrestrial ²⁵Mg/²⁴Mg value of 0.12663 determined by Catanzaro *et al.*²⁵. This value is typical of the ²⁵Mg/²⁴Mg ratios measured by SHRIMP on terrestrial hibonite to within $\pm 2\%$ per AMU. Instrumentally induced fractionation is therefore negligible in determining the intrinsic Mg fractionation of 7-971 compared with the uncertainties of the individual analyses derived from counting statistics. The residual is the relative deviation of the normalized ²⁶Mg/²⁴Mg ratio, expressed in per mil (‰), from the terrestrial value of 0.139432 (± 13) as measured on SHRIMP²³. The normalization removes the effects of mass-dependent fractionation, both analytical and intrinsic to the target. A linear approximation to the power law is sufficiently accurate for the treatment here.

Owing to the very high ²⁷Al/²⁴Mg ratio, the ²⁷Al⁺ was too intense to be measured on the ion counter during the Mg isotope measurements. The ²⁷Al/²⁴Mg ratios were estimated from the ²⁴Mg⁺ count rates and standardized using the ²⁷Al⁺/²⁴Mg⁺ ratio of area 1 (after the completion of core analysis 1-2), along with

those of three other meteoritic hibonites. The $^{27}\text{Al}^{+}/^{24}\text{Mg}^{+}$ ratios of analysis 1-2 and the standard hibonites were determined separately at a lower secondary ion intensity by reducing the primary beam intensity. The measured $^{27}\text{Al}^{+}/^{24}\text{Mg}^{+}$ for analysis 1-2 was calibrated against the other hibonites whose atomic Al/Mg ratios had been measured by an electron microprobe. As these hibonites are all approximately 90% Al_2O_3 , the Al/Mg ratio is controlled primarily by the Mg content. Therefore this calibration depends on the assumption that the Al^{+} intensity and the $(\text{Al}^{+}/\text{Mg}^{+})/(\text{Al}/\text{Mg})$ did not change during the course of the analyses. A small fraction of the secondary ion beam was collected on the secondary beam monitor (SBM) before the source slit²³. The counts registered on the SBM were uniform during the course of the analyses suggesting that the intensity of Al^{+} , which is the dominant component of the secondary ion beam (>80%), was also stable. Variation in the relative efficiency for Al^{+} and Mg^{+} is unlikely to cause the large range in $^{27}\text{Al}^{+}/^{24}\text{Mg}^{+}$ as variations reported from a wide range of matrices have differed by only ~15%^{5-8,19}. The Al/Mg calibration could be affected by variations in the secondary ion extraction parameters and may be systematically in error by as much as 50%. However, this error should be nearly constant for all analyses of 7-971, because the extraction parameters are relatively fixed for a single crystal of this size.

Table 1 shows the data obtained from two areas within 7-971 in chronological order. The first area analysed was near the centre of the grain. The $^{24}\text{Mg}^{+}$ current immediately rose as the primary ion beam cut through the carbon coating, but then decayed. This was due to contamination by magnesium smeared over the surface of the mount from surrounding olivine grains during polishing, because the same $^{24}\text{Mg}^{+}$ rise and decay was observed when the beam was placed on the epoxy mounting-medium. The data for analysis 1-1 were collected after the primary beam had largely cut through the carbon coat and the counts collected from the Mg isotopes all show a systematic exponential decay with time (Fig. 1). But the SBM indicates that the secondary ion beam is stable and therefore the Al^{+} intensity is also probably stable. All ratios show a systematic temporal increase as the surface Mg is removed and the ratios define linear mixing arrays from near terrestrial Mg isotopic compositions, at low values of $^{27}\text{Al}^{+}/^{24}\text{Mg}^{+}$, to isotopically exotic Mg at high values of $^{27}\text{Al}^{+}/^{24}\text{Mg}^{+}$ (Fig. 2). Isotope ratios were calculated using a linear interpolation for intensity variations. This was inadequate for the dramatic exponential decay in analysis 1-1 and a logarithmic interpolation was used.

The primary beam was left on the hibonite for about 30 min, until the $^{24}\text{Mg}^{+}$ intensity had stabilized, and then 30 scans were recorded (analysis 1-2). The data have a mean $\delta^{26}\text{Mg}$ of $3,550(\pm 60)\%$ with values up to $3,860(\pm 100)\%$ at $^{27}\text{Al}^{+}/^{24}\text{Mg}^{+}$ of $\sim 6 \times 10^4$. This requires a maximum initial $^{26}\text{Al}/^{27}\text{Al}$ ratio of only 9×10^{-6} to account for the excess ^{26}Mg in this crystal. The mean F is also above the terrestrial ratio at $+92(\pm 10)\%$ per AMU. Isotopically heavy Mg was also recorded from the Dhajala hibonite, which had a mean F of $+19\%$ per AMU²¹, but no anomalous fractionation has been reported for HAL.

The second analysis was from a location nearer to the rim of the grain and reproduces the effects seen in the first analysis. Again the $^{24}\text{Mg}^{+}$ intensity decayed as the surface contamination was removed, although the $^{24}\text{Mg}^{+}$ stabilized at a slightly higher count rate, and hence at a lower value of $^{27}\text{Al}^{+}/^{24}\text{Mg}^{+}$ than the first area. However the mean $\delta^{26}\text{Mg}$ for this analysis is markedly lower at $1,450(\pm 60)\%$, the highest value being $1,650(\pm 70)\%$. The fractionation recorded for this second area is also heavy relative to terrestrial values with a mean value of $28(\pm 13)\%$ per AMU, although individual analyses range from $-44 \pm 50\%$ to $+129 \pm 58\%$.

The systematic temporal increase of $^{27}\text{Al}^{+}/^{24}\text{Mg}^{+}$ and $\delta^{26}\text{Mg}$ during the analyses suggests that terrestrial contamination is probably significant in analyses 1-2 and 2-1. This could be due to a faint halo of scattered ions around the primary beam, or

Table 1 Magnesium isotope data for hibonite 7-971

$^{27}\text{Al}/^{24}\text{Mg}$	$^{25}\text{Mg}/^{24}\text{Mg}$	$^{26}\text{Mg}/^{24}\text{Mg}$	$F^{\dagger}$ (% per AMU)	$\delta^{26}\text{Mg}^*$ (%)
Analysis 1-1				
8,600 ± 30	0.127 ± 0.001	0.217 ± 0.002	5 ± 11	546 ± 26
14,500 ± 70	0.132 ± 0.002	0.262 ± 0.003	44 ± 15	791 ± 36
18,880 ± 110	0.132 ± 0.002	0.302 ± 0.004	44 ± 17	1,076 ± 44
24,700 ± 160	0.135 ± 0.003	0.340 ± 0.004	63 ± 20	1,316 ± 51
29,000 ± 210	0.138 ± 0.003	0.377 ± 0.005	92 ± 22	1,520 ± 58
32,600 ± 250	0.138 ± 0.003	0.413 ± 0.006	90 ± 23	1,783 ± 63
34,900 ± 270	0.137 ± 0.003	0.429 ± 0.006	84 ± 24	1,908 ± 66
36,900 ± 300	0.142 ± 0.003	0.447 ± 0.007	120 ± 25	1,964 ± 69
38,100 ± 310	0.139 ± 0.003	0.460 ± 0.007	100 ± 26	2,099 ± 70
39,900 ± 300	0.139 ± 0.003	0.485 ± 0.007	100 ± 26	2,275 ± 73
Analysis 1-2				
46,900 ± 430	0.141 ± 0.004	0.616 ± 0.009	113 ± 29	3,191 ± 87
47,400 ± 430	0.140 ± 0.004	0.623 ± 0.009	106 ± 29	3,257 ± 87
48,800 ± 450	0.143 ± 0.004	0.618 ± 0.009	129 ± 30	3,174 ± 89
49,400 ± 460	0.140 ± 0.004	0.645 ± 0.010	106 ± 29	3,415 ± 90
49,300 ± 460	0.135 ± 0.004	0.649 ± 0.010	66 ± 29	3,522 ± 90
51,100 ± 480	0.137 ± 0.004	0.658 ± 0.010	82 ± 29	3,555 ± 92
52,800 ± 510	0.137 ± 0.004	0.633 ± 0.010	82 ± 30	3,376 ± 92
53,000 ± 512	0.139 ± 0.004	0.647 ± 0.010	98 ± 30	3,445 ± 94
53,100 ± 510	0.135 ± 0.004	0.635 ± 0.010	66 ± 30	3,422 ± 92
52,900 ± 510	0.140 ± 0.004	0.636 ± 0.010	106 ± 30	3,350 ± 93
53,300 ± 520	0.133 ± 0.004	0.652 ± 0.010	50 ± 30	3,576 ± 93
53,300 ± 520	0.140 ± 0.004	0.667 ± 0.010	106 ± 30	3,572 ± 95
54,700 ± 540	0.136 ± 0.004	0.645 ± 0.010	74 ± 30	3,478 ± 94
54,500 ± 530	0.139 ± 0.004	0.654 ± 0.010	98 ± 31	3,495 ± 95
54,600 ± 530	0.137 ± 0.004	0.665 ± 0.010	82 ± 30	3,606 ± 96
55,000 ± 540	0.135 ± 0.004	0.676 ± 0.010	66 ± 30	3,716 ± 96
56,900 ± 570	0.140 ± 0.004	0.675 ± 0.011	106 ± 31	3,630 ± 99
55,500 ± 550	0.134 ± 0.004	0.653 ± 0.010	58 ± 30	3,567 ± 95
55,600 ± 550	0.135 ± 0.004	0.669 ± 0.010	66 ± 30	3,666 ± 96
57,500 ± 580	0.142 ± 0.004	0.691 ± 0.011	121 ± 31	3,713 ± 101
57,900 ± 580	0.147 ± 0.004	0.680 ± 0.011	161 ± 33	3,555 ± 101
58,000 ± 580	0.142 ± 0.004	0.690 ± 0.011	121 ± 32	3,706 ± 101
58,100 ± 590	0.136 ± 0.004	0.667 ± 0.011	74 ± 31	3,636 ± 99
57,100 ± 570	0.135 ± 0.004	0.680 ± 0.011	66 ± 31	3,745 ± 98
57,600 ± 580	0.138 ± 0.004	0.686 ± 0.011	90 ± 31	3,740 ± 100
57,800 ± 580	0.140 ± 0.004	0.686 ± 0.011	106 ± 32	3,709 ± 100
59,500 ± 610	0.139 ± 0.004	0.666 ± 0.011	98 ± 32	3,581 ± 100
58,400 ± 590	0.139 ± 0.004	0.679 ± 0.011	98 ± 32	3,674 ± 100
57,000 ± 570	0.136 ± 0.004	0.678 ± 0.011	74 ± 31	3,715 ± 98
59,200 ± 600	0.138 ± 0.004	0.703 ± 0.011	90 ± 32	3,862 ± 102
Analysis 2-1				
27,000 ± 190	0.124 ± 0.003	0.271 ± 0.004	-21 ± 20	985 ± 50
32,100 ± 240	0.127 ± 0.003	0.294 ± 0.005	3 ± 22	1,103 ± 56
35,500 ± 280	0.127 ± 0.003	0.306 ± 0.005	3 ± 24	1,189 ± 59
38,000 ± 310	0.134 ± 0.003	0.327 ± 0.005	58 ± 25	1,229 ± 63
38,800 ± 320	0.130 ± 0.003	0.333 ± 0.006	27 ± 25	1,335 ± 64
38,600 ± 320	0.127 ± 0.003	0.340 ± 0.006	3 ± 25	1,433 ± 63
38,900 ± 320	0.126 ± 0.003	0.317 ± 0.005	-5 ± 25	1,283 ± 62
40,100 ± 340	0.125 ± 0.003	0.339 ± 0.006	-13 ± 25	1,457 ± 64
41,500 ± 350	0.126 ± 0.003	0.338 ± 0.006	-5 ± 25	1,434 ± 65
41,400 ± 350	0.121 ± 0.003	0.354 ± 0.006	-44 ± 25	1,628 ± 65
42,300 ± 360	0.126 ± 0.003	0.340 ± 0.006	-5 ± 26	1,448 ± 66
42,000 ± 360	0.128 ± 0.003	0.355 ± 0.006	11 ± 26	1,524 ± 67
44,800 ± 400	0.135 ± 0.004	0.357 ± 0.006	66 ± 27	1,428 ± 70
44,500 ± 390	0.137 ± 0.004	0.361 ± 0.006	82 ± 27	1,425 ± 71
45,300 ± 400	0.135 ± 0.004	0.356 ± 0.006	66 ± 28	1,421 ± 71
43,800 ± 380	0.126 ± 0.003	0.363 ± 0.006	-5 ± 26	1,613 ± 68
45,600 ± 410	0.132 ± 0.004	0.365 ± 0.006	42 ± 27	1,533 ± 71
45,400 ± 410	0.131 ± 0.003	0.349 ± 0.006	35 ± 27	1,434 ± 70
44,300 ± 390	0.131 ± 0.003	0.370 ± 0.006	35 ± 27	1,585 ± 70
46,600 ± 420	0.143 ± 0.004	0.384 ± 0.007	129 ± 28	1,495 ± 75
45,000 ± 400	0.135 ± 0.004	0.351 ± 0.006	66 ± 27	1,385 ± 70
45,500 ± 410	0.131 ± 0.003	0.374 ± 0.006	35 ± 27	1,613 ± 71
47,100 ± 430	0.129 ± 0.004	0.366 ± 0.006	19 ± 27	1,588 ± 72
45,900 ± 410	0.131 ± 0.003	0.364 ± 0.006	35 ± 27	1,542 ± 71
46,400 ± 420	0.128 ± 0.003	0.372 ± 0.006	11 ± 27	1,646 ± 71
46,700 ± 420	0.136 ± 0.004	0.383 ± 0.007	74 ± 28	1,599 ± 73
47,100 ± 430	0.132 ± 0.004	0.368 ± 0.006	42 ± 28	1,554 ± 72
47,000 ± 430	0.131 ± 0.004	0.359 ± 0.006	35 ± 28	1,506 ± 71
48,600 ± 450	0.133 ± 0.004	0.372 ± 0.007	50 ± 28	1,567 ± 74
47,800 ± 440	0.129 ± 0.004	0.368 ± 0.007	19 ± 28	1,602 ± 72

All errors $\pm 1\sigma$ (standard deviation).

* $\delta^{26}\text{Mg} = [(^{26}\text{Mg}/^{24})/0.139432 - 1] \times 1,000 - 2F$.

† $F = [(^{25}\text{Mg}/^{24}\text{Mg})/0.12663 - 1]/1,000$.

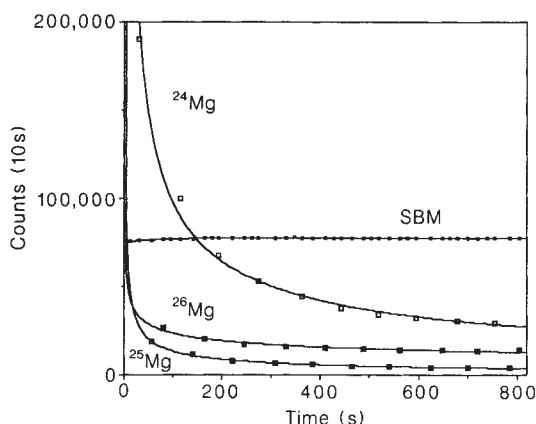


Fig. 1 Exponential decay with time of Mg-isotope count rates from analysis 1-1 as primary beam cuts through surface contamination. The constancy of the secondary beam monitor (SBM) counts is indicative of Al^+ stability. Lines through Mg counts are least-squares logarithmic regressions.

'knock on' of surface Mg into the sample. Consequently $\delta^{26}\text{Mg}$ and F values should be regarded as minimum values. Similarly the mean values for $\delta^{26}\text{Mg}$ and F are not derived from a normal distribution and the errors of the means are not indicative of the range in the data.

As well as the difference between the compositions of the two areas, the hibonite is heterogeneous on a microscale. This is demonstrated by the projection of the $\delta^{26}\text{Mg}$ array of analysis 1-1 which does not pass through analysis 1-2 and indicates a distinct change in the character of the material being sputtered. These data cannot be due to simple mixing of isotopically normal Mg and an exotic Mg component. The Mg isotope systematics can, of course, be described by a mixture of three components. The simplest and most flexible description would incorporate normal Mg, pure ^{26}Mg and mass-fractionated Mg with F of $\sim +150\%$ per AMU.

The two exotic components can be readily produced. A pure ^{26}Mg component can be obtained from the decay of ^{26}Al , either *in situ* or by chemical memory of fossil ^{26}Mg from ^{26}Al decay before hibonite formation. Mass-fractionated Mg could be produced from Rayleigh distillation in which the heavier isotopes are enriched in the residue. But the chemical memory model could be extended to include an exotic composition which is anomalous in $^{25}\text{Mg}/^{24}\text{Mg}$ as well as $^{26}\text{Mg}/^{24}\text{Mg}$. A unique solution to the possible processes and resulting components that have contributed to the Mg isotopic composition of 7-971 is not possible.

A unique feature of this hibonite is the large difference in excess ^{26}Mg between the two analysed areas. If the excesses are due to *in situ* decay of ^{26}Al , then it suggests that the $^{26}\text{Al}/^{27}\text{Al}$ changed during the course of hibonite formation. This requires spatial heterogeneity of the local area in which the hibonite formed, or a prolonged period of crystallization over several hundred thousand years during which the $^{26}\text{Al}/^{27}\text{Al}$ ratio of the gas decayed. In a chemical memory model, isotope heterogeneities may be preserved if the hibonite was never completely molten but is a sintered aggregate.

It is possible that the Mg isotope systematics of 7-971 are not pristine but have been disturbed during a later heating event. The hibonite originally may have had a uniform enrichment of ^{26}Mg from either *in situ* decay of ^{26}Al , or fossil ^{26}Mg , which has been modified during preferential evaporation of Mg. Distillation would leave the residual Mg isotopically heavy by mass fractionation and this may be the source of the high $^{25}\text{Mg}/^{24}\text{Mg}$ ratios in the hibonite. Another possible signature of distillation is the characteristic rare-earth element patterns of the Dhajala²⁶, HAL²⁷, and 7-971²⁸ hibonites, which all show substantial cerium depletions as a distinctive feature.

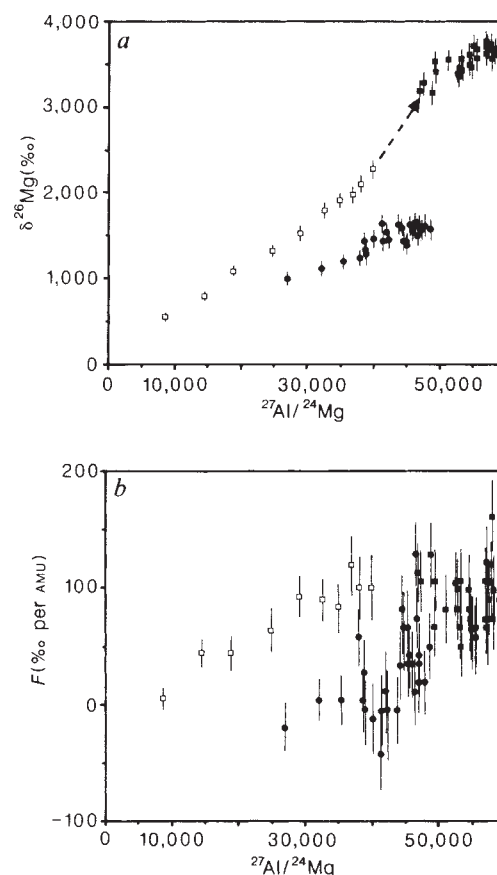


Fig. 2 *a*, Residual $\delta^{26}\text{Mg}$ and *b*, fractionation F plotted against $^{27}\text{Al}/^{24}\text{Mg}$. Two locations in 7-971 were analysed, analyses 1-1 and 1-2 were taken near the centre of the crystal whereas analysis 2-1 was taken closer to the rim. Both F and $\delta^{26}\text{Mg}$ in analysis 1-1 show mixing lines between near-normal isotopic Mg (which is a surface contaminant) at low $^{27}\text{Al}/^{24}\text{Mg}$, and exotic Mg at high $^{27}\text{Al}/^{24}\text{Mg}$ after most of the surface Mg has been removed (see Fig. 1). Data were taken for analysis 1-2 after the count rate had stabilised. The mean excess $\delta^{26}\text{Mg}$ for analysis 1-2 is 3550 ± 60 (2σ)‰ with a maximum value of $3860 \pm 100\%$. Analysis 2-1 shows much lower excesses with a mean $\delta^{26}\text{Mg}$ of $1550 \pm 60\%$ and a maximum of $1650 \pm 70\%$. F is also above the terrestrial value for both analyses with mean values of $+92 \pm 10\%$ per AMU for analysis 1-2 and $+28 \pm 13\%$ per AMU for analysis 2. (1σ error bars are shown as calculated from counting statistics for F and $\delta^{26}\text{Mg}$; the error for $^{27}\text{Al}/^{24}\text{Mg}$ is approximately the same size as the symbol. Symbols: open squares, analysis 1-1; closed squares, analysis 1-2; circles, analysis 2-1.)

Distillation will not affect the $\delta^{26}\text{Mg}$ for any localized area in the hibonite provided the various Mg isotope components are mobilized together. However, if the exotic Mg in the hibonite is subsequently mixed with isotopically normal Mg, then $\delta^{26}\text{Mg}$ and F will be reduced. Mg uptake, and hence dilution of the exotic Mg, will be greatest at the edge of the hibonite owing to diffusion processes. The Al/Mg ratio of the rim would be raised by distillation but lowered by normal Mg infusion. The source of the normal Mg could be the ambient gas in the solar nebula, as proposed by Clayton¹¹, or it could be related to metamorphism of the hibonite in the meteorite. The higher extent of mixing of normal Mg observed in the Dhajala hibonite and HAL could be related to the higher degree of metamorphism in the Dhajala (H3) and Allende (C3V) chondrites compared with the Murchison (C2M) chondrite.

Hibonite 7-971 preserves the largest excesses in ^{26}Mg yet reported, however the excess ^{26}Mg is locally heterogeneous. The Mg isotope systematics of this hibonite have probably been

disturbed in a thermal event which also produced isotopically heavy Mg from mass-dependent fractionation. It is not possible to uniquely identify the possible ^{26}Mg contributions from *in situ* decay of ^{26}Al or chemical memory of exotic Mg.

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1. Lee, T., Papanastassiou, D. A. & Wasserburg, G. J. *Astrophys. J.* **211**, L107-L110 (1977).
2. Stegmann, W. & Begemann, F. *Earth planet. Sci. Lett.* **55**, 266-272 (1981).
3. Hucheeon, J. D., Armstrong, J. T. & Wasserburg, G. J. *Lunar planet. Sci. XVII*, 372-373 (1986).
4. Lorin, J. C. *et al. Meteoritics* **12**, 299-300 (1977).
5. Bradley, J. G., Huneke, J. C. & Wasserburg, G. J. *J. geophys. Res.* **83**, 244-254 (1978).
6. Hucheeon, I. D. *et al. Geochim. cosmochim. Acta Suppl.* **9**, 1345-1368 (1978).
7. Hucheeon, I. D. in *Nuclear and Chemical Dating Techniques: Interpreting the Environmental Record* (ed. Currie, L. A.) 95-128 (American Chemical Society Symposium Series No. 176, 1982).
8. Huneke, J. C., Armstrong, J. T. & Wasserburg, G. J. *Geochim. cosmochim. Acta* **47**, 1635-1650 (1983).
9. Mahoney, W. A. *et al. Astrophys. J.* **286**, 578-585 (1984).
10. Clayton, D. D. *Astrophys. J.* **199**, 765-769 (1975).
11. Clayton, D. D. *Astrophys. J.* **310**, 490-498 (1986).
12. MacDougall, J. D. & Phinney, D. *Geophys. Res. Lett.* **6**, 215-218 (1979).
13. Hucheeon, I. D. *et al. Meteoritics* **15**, 306-307 (1980).
14. Papanastassiou, D. A. & Wasserburg, G. J. *Meteoritics* **15**, 348-349 (1980).
15. Bar-Matthews, M. *et al. Geochim. cosmochim. Acta* **46**, 31-41 (1982).
16. Hucheeon, I. D. *et al. Lunar planet. Sci. XIV*, 339-340 (1983).
17. Hinton, R. W., Grossman, L. & MacPherson, G. J. *Meteoritics* **19**, 240-241 (1984).
18. Hinton, R. W., Davis, A. M. & Scatena-Wachel, D. E., *Lunar planet. Sci. XVI*, 354-355 (1985).
19. Fahey, A. J. *et al. Lunar planet. Sci. XVI*, 227-228 (1985).
20. Hinton, R. W. & Davis, A. M. *Lunar planet. Sci. XVII*, 344-345 (1986).
21. Hinton, R. W. & Bischoff, A. *Nature* **308**, 169-172 (1984).
22. Fahey, A. J. *et al. Geochim. cosmochim. Acta* **51**, 329-350 (1987).
23. Ireland, T. R., Compston, W. & Esat, T. M. *Geochim. cosmochim. Acta* **50**, 1413-1422 (1986).
24. Lee, T., Russell, W. A. & Wasserburg, G. J. *Astrophys. J.* **228**, L93-L98 (1979).
25. Catanzaro, E. J., Murphy, T. J., Garner, E. L. & Shields, W. R. *J. Res. NBS* **70A**, 453-458 (1966).
26. Hinton, R. W., Davis, A. M. & Scatena-Wachel, D. E. *Lunar planet. Sci. XVI*, 352-353 (1985).
27. Davis, A. M. *et al. Geochim. cosmochim. Acta* **46**, 1627-1651 (1982).
28. Ireland, T. R. *et al. Meteoritics* **21**, 404-405 (1987).

Evidence for liquid immiscibility in the upper mantle

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Liquid immiscibility, the separation of magmas into two or more immiscible liquid phases, has been shown to occur both experimentally¹⁻³ and in nature^{4,5}, but this phenomenon has received only limited attention in modelling magmatic processes, as known cases of liquid immiscibility are generally restricted to magma compositions encountered during late stages of crystal fractionation, and relatively shallow crustal levels. Here I report the results of a study of trapped quenched liquids in spinel lherzolite xenoliths, these are fragments of upper mantle material carried to the surface in volcanic rocks. These xenoliths carry quenched coexisting basaltic, carbonatitic and volatile-rich ultramafic liquids, showing the existence of a previously unknown field of three-liquid immiscibility. This type of immiscibility occurs in primitive volatile-rich magma compositions at upper mantle to crustal levels. Furthermore, metasomatic growth of amphibole $\pm$ apatite $\pm$ phlogopite in the lherzolites is associated with introduction of carbonatitic and volatile-rich ultramafic liquids, indicating that such liquids may play an important role in mantle metasomatism.

Quaternary eruptions of nepheline basanites on North-west (NW) Spitsbergen, associated with a regional graben structure, have been related to activity on a nearby hotspot and on plate boundaries⁶⁻⁸. These volcanic centres are probably the world's richest occurrence of upper mantle and lower crustal xenoliths. Xenolith pressure, P , and temperature, T , data indicate a thin (27 km) continental crust and high geothermal gradient (9 kbar/950 °C-17 kbar/1,150 °C)⁹.

The spinel lherzolite xenoliths contain glasses and microcrystalline aggregates of carbonates, showing three different modes of occurrence: (1) in cross-cutting fractures penetrating the lherzolites; (2) associated with incipient fusion of the lherzolite

Table 1 Representative glass analyses

	Host basanite 1	In fracture 2 3		Associated with incipient fusion 4 5 6			Inclusions 7 8	
SiO ₂	44.77	55.46	57.97	49.23	2.92	53.57	57.77	53.28
TiO ₂	1.97	2.30	0.03	3.02	0.04	0.04	0.01	0.11
Al ₂ O ₃	13.10	17.86	0.08	20.07	0.37	0.07	0.35	4.67
Cr ₂ O ₃	0.07	0.02	0.02	0.04	0.00	0.01	0.02	0.12
FeO ¹	9.84	3.94	3.93	6.01	1.92	6.34	6.46	8.92
MnO	0.18	0.17	0.07	n.a.	n.a.	n.a.	0.07	0.11
MgO	12.75	3.41	26.89	4.81	44.03	22.56	25.10	21.36
NiO	0.06	0.05	0.22	0.03	0.19	0.50	0.15	2.10
CaO	7.72	6.76	0.38	11.06	0.34	0.53	0.51	1.01
Na ₂ O	5.08	5.59	0.07	3.40	0.04	0.12	0.02	0.06
K ₂ O	1.89	2.12	0.08	0.53	0.04	0.15	0.13	0.28
P ₂ O ₅	0.87	0.73	0.09	0.00	0.00	0.00	0.00	0.00
Total	98.30	98.41	89.83	98.20	49.89	83.91	86.67	92.02
100Mg/(Mg + Fe ¹)	69.8	60.7	92.4	58.9	97.6	86.4	87.4	81.0

The column numbers indicate: 1, most primitive host basanite; 2, silica-rich basaltic glass in fracture in spinel lherzolite (Fig. 1a); 3, ultramafic glass with carbonate spherules, occurring as droplets in (2); 4, basaltic glass associated with incipient fusion of spinel lherzolite (Fig. 1c); 5, magnesitic carbonatite glass, occurring as droplets in basaltic glass associated with incipient fusion of spinel lherzolite (Fig. 1d); 6, ultramafic glass with carbonate spherules, occurring as droplets in (4); 7, inclusion of ultramafic glass in pargasitic amphibole replacing spinel (Fig. 1g); 8, inclusion of ultramafic glass in olivine adjacent to amphibole-vein. Basanite composition obtained by X-ray fluorescence analysis, Macquarie University, New South Wales, Australia. Glass compositions obtained by electron microprobe analysis, Mineralogisk-Geologisk Museum, Oslo, Norway.

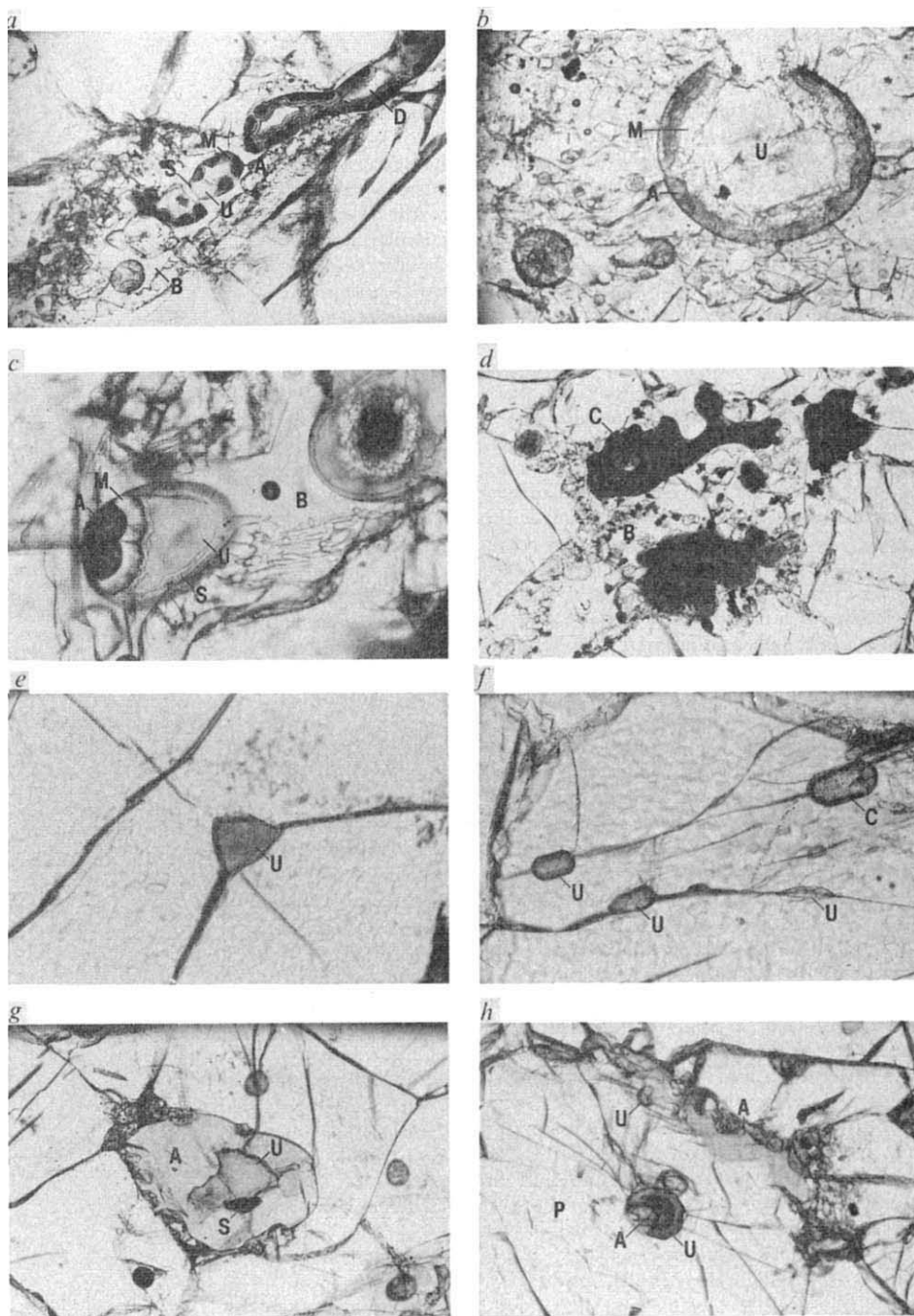
assemblage; and (3) occurring interstitially and as inclusions in minerals in amphibole $\pm$ apatite $\pm$ phlogopite-bearing lherzolites.

Silica-rich basaltic glass (Table 1) occurs in some lherzolites, in penetrating fractures extending from the surrounding host basanite, and along grain boundaries. This liquid contains amounts of Ti, Na, K and P similar to the host basanite, and is associated with incipient incongruent melting of enstatite and outplating of olivine and spinel. Abundant droplets of two different types, bounded by sharp menisci, occur in the glass (Fig. 1a). The first type consists mainly of dolomite; the second type consists mainly of a brownish ultramafic glass (Table 1), showing a faint cross-hatched or spotted appearance in polarized light. In addition, both types of droplets are partially surrounded by a thin rim of silica and carry composite spherules of ankerite/dolomite and magnesite. Microprobe analysis of the ultramafic glass gives very low totals, indicating the presence of large amounts of volatiles (10-17 wt%), probably H₂O $\pm$ CO₂. Trace amounts of both types of droplets also occur in the host basanites (Fig. 1b).

Basaltic glass associated with incipient fusion of the lherzolite assemblage (Table 1) typically surround corroded spinels, or is associated with incipiently melted amphibole or phlogopite. These melts have variable compositions, but differ from the silica-rich basaltic liquid by having higher Al and Ca and no P. Droplets with carbonates and/or droplets with ultramafic glass (Fig. 1c) commonly occur in these melts. Droplets of carbonate material are similar to those described above, but are in rare cases present as quenched magnesitic carbonatite glass (Fig. 1d) (Table 1). Compositions of ultramafic glass in droplets (Table 1) are also similar to those described above, though droplets of ultramafic glass in some samples do not carry carbonates and contain minor Al, Ca, K and up to 1 wt% NiO.

Pargasitic amphibole $\pm$ apatite $\pm$ phlogopite occur in some lherzolites, either evenly distributed or concentrated in amphibole-rich veins. Carbonates and/or ultramafic glass are particularly abundant adjacent to amphibole-veins, occurring both interstitially as an integral part of the metamorphic microtexture (Fig. 1e), and as rounded inclusions in lherzolite minerals and in amphibole (Fig. 1f, g) associated with fluid

Fig. 1 *a*, Silica-rich basaltic glass (B), carrying droplets of dolomite (D) and volatile-rich ultramafic glass (U), in fracture in spinel lherzolite. Both types of droplets are partially surrounded by a thin rim of silica (S), and carry spherules of ankerite/dolomite (A)+magnesite (M). Width of field 1.3 mm. *b*, Droplet of volatile-rich ultramafic glass (U) in host basanite. Abbreviations as for (a). Width of field 0.35 mm. *c*, Droplets of volatile-rich ultramafic glass (U) in basaltic glass (B) in incipiently melted spinel lherzolite. Abbreviations as for (a). Width of field 0.35 mm. *d*, Droplets of magnesitic carbonatite glass (C) in basaltic glass (B) in incipiently melted spinel lherzolite. Width of field 1.3 mm. *e*, Volatile-rich ultramafic glass (U) occurring interstitially between olivine grains in spinel lherzolite. Width of field 0.65 mm. *f*, Microcrystalline carbonate (C) and volatile-rich ultramafic glass (U) occurring interstitially, and as inclusions in enstatite, adjacent to amphibole-vein in spinel lherzolite. Width of field 0.65 mm. *g*, Volatile-rich ultramafic glass (U) as inclusion in pargasitic amphibole (A) replacing spinel (S) adjacent to amphibole-vein. Width of field 1.3 mm. *h*, Inclusions of volatile-rich ultramafic glass (U) in Cr-diopside (P) partially replaced by pargasitic amphibole (A) adjacent to amphibole-vein. Note euhedral pargasite daughter crystal in large inclusion. Width of field 1.3 mm.



inclusions of high-density CO_2 . Carbonates generally occur as composite spheroidal aggregates consisting of ankerite/dolomite and magnesite, but are in rare cases present as magnesite or calcite only. The ultramafic glass is similar to ultramafic glass in droplets described above, but rarely carries carbonates. Ultramafic glass showing this mode of occurrence has nearly constant composition, and contains minor Al, Ca, K and generally around 0.2–0.3 wt% NiO (Table 1). One inclusion of ultramafic glass in olivine was found to contain as much as 2.1 wt% NiO (Table 1). Sulphur, which could indicate the presence of Ni-rich sulphides, was not detected. One inclusion of ultramafic glass in clinopyroxene was found to carry a daughter crystal of pargasitic amphibole (Fig. 1h).

The carbonates and carbonatitic glass described above are interpreted to represent quenched carbonatitic liquid. Similarly, the ultramafic glass is interpreted to represent quenched volatile-

rich ultramafic liquid. The sharp menisci separating the quenched coexisting basaltic, carbonatitic and ultramafic liquids indicate the existence of a previously unknown field of three-liquid immiscibility, occurring in primitive volatile-rich magma compositions. Quenched carbonatitic and ultramafic liquids occurring both as immiscible droplets in the host basanites and as separate inclusions in lherzolite minerals, indicate that this three-liquid field may exist at magmatic temperatures under both crustal and upper mantle pressures.

Major and minor elements and volatiles are strongly fractionated among coexisting liquids. Ti, Al, Na, K and P are partitioned into the basaltic liquid; Mg, Fe and Ca seem to show variable partitioning into the carbonatitic liquid; Mg, Ni and volatiles are partitioned into the ultramafic liquid. The strong partitioning of compatible elements, and very low partitioning of incompatible elements, into the ultramafic liquid indicates

the presence of a melt structure different from that occurring in normal silicate melts. Carbonate spherules occurring in immiscible droplets of ultramafic liquid in some samples may indicate closure of the three-liquid field along the boundary between carbonatitic and ultramafic liquid, probably with increasing temperature.

Silicate-silicate immiscibility has been documented elsewhere in naturally occurring magma compositions, generally resulting in coexisting felsic and mafic liquids^{4,5}. The silicate-silicate immiscibility described here differs from other known cases in that it occurs in primitive volatile-rich magma compositions, and results in immiscible basaltic and volatile-rich ultramafic liquids. Compositions of coexisting pairs of basaltic and ultramafic glass are plotted in the pseudoternary Greig diagram of Weiblen and Roedder¹⁰ in Fig. 2a.

Carbonate-silicate immiscibility has been documented experimentally only in systems with very high alkali-oxide concentrations^{11,12}, for example resulting in immiscible alkali-carbonatite and phonolite³. Quenched coexisting basaltic and carbonatitic glass analysed in this study show that carbonate-silicate immiscibility is not necessarily dependent on very high alkali-oxide concentrations, and may result in immiscible alkali-free carbonatite liquid (Table 1).

The field of three-liquid immiscibility, defined by coexisting basaltic, carbonatitic and volatile-rich ultramafic liquids, is shown in the pseudoternary Greig diagram in Fig. 2b. Here, the constructed three-liquid field covers a range of magma compositions, including carbonatites, kimberlites, ultramafic lamproites and some alkaline basalts.

The occurrence of the silica-rich basaltic liquid in fractures extending from the surrounding host basanite, together with the observed melting of enstatite in contact with this liquid, suggests that it represents basanite infiltrating the lherzolites shortly before or during eruption. The similarity between this liquid and the host basanites with regard to the amount of Ti, Na, K and P present supports this interpretation, though reactions with the lherzolite make direct comparisons with the host basanites difficult. If this liquid represents basanite infiltrating the xenolith material, the basanite must at an early stage have carried a significant amount of immiscible carbonatitic and volatile-rich ultramafic liquids. Sparse immiscible droplets of these liquids occurring in the host basanites support this possibility, indicating that gravitational separation of immiscible liquids may have occurred during eruption.

Introduction of a three-liquid assemblage, consisting of basaltic, carbonatitic and ultramafic liquids, may also explain the association of trapped carbonatitic and ultramafic liquids with amphibole-rich veins in some lherzolites. Mass-balance calculations, combined with the constructed immiscibility field (Fig. 2c), show that crystallization mainly of amphibole from such a liquid assemblage may lead to residual carbonatitic and ultramafic liquids only. During crystallization of amphibole in veins, the carbonatitic and volatile-rich ultramafic liquids may have been forced into the lherzolite matrix, promoting metasomatic growth of amphibole at the expense of lherzolite phases. Thus, trapped carbonatitic and ultramafic liquids in these lherzolites may represent the actual fluids that were associated with metasomatic alteration of the lherzolite material.

It seems unlikely that the assemblage of liquids, inferred to have infiltrated the lherzolites, could have migrated any significant distance without separating. This suggests the existence of a homogeneous protoliquid, which unmixed into immiscible basaltic, carbonatitic and volatile-rich ultramafic liquids at some stage during upward migration. An alternative explanation would be that the basaltic liquid mixed with carbonatitic and ultramafic liquids residing in the lherzolites. However, the presence of trapped carbonatitic and ultramafic liquids seems itself to be associated with introduction of a basaltic component, as represented by the amphibole-rich veins. Reconstruction of the protoliquid, by reintegrating the liquid assemblages infiltrating

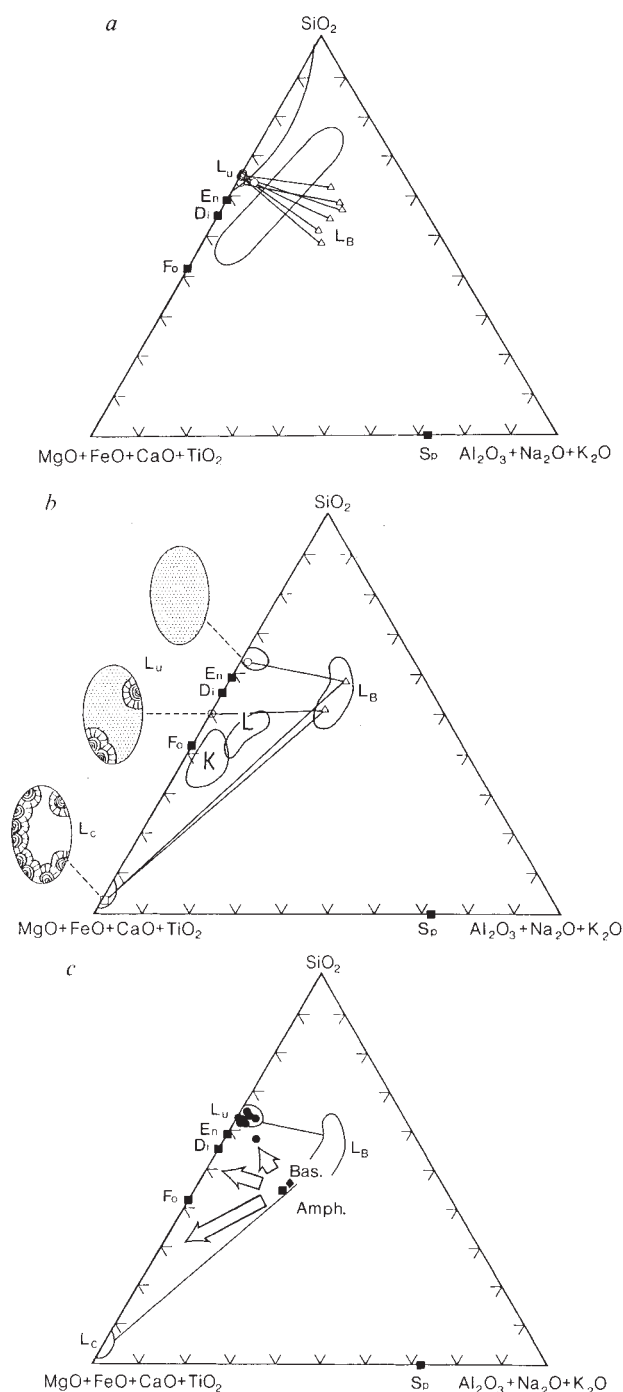
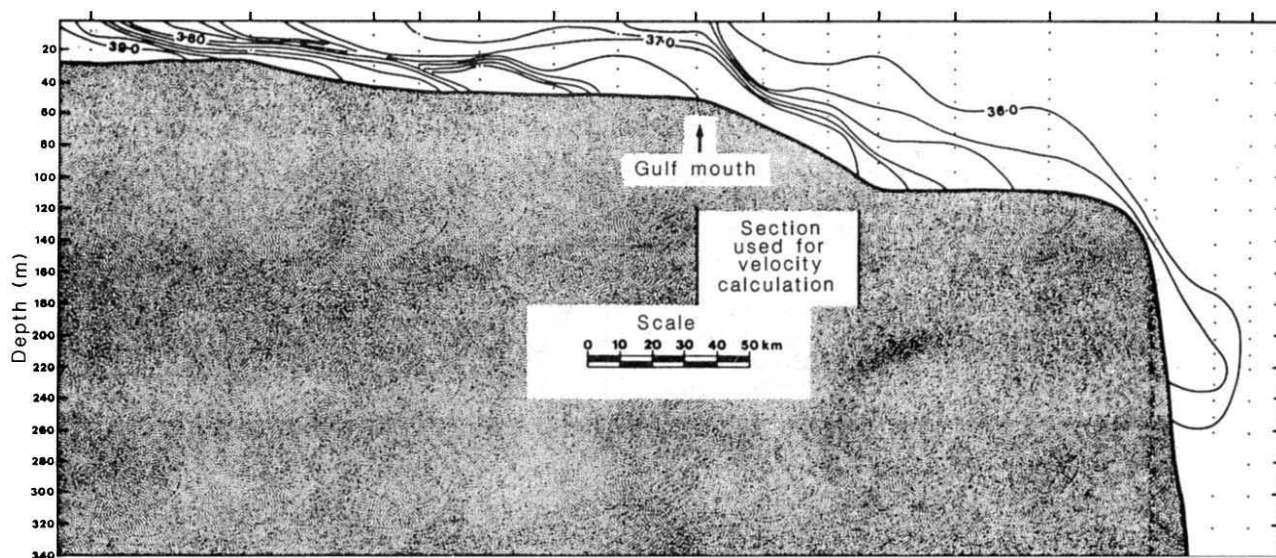


Fig. 2 *a*, Coexisting pairs of basaltic (L_B) and volatile-rich ultramafic glass (L_U) plotted in the pseudoternary Greig diagram. L_B represents compositions of both silica-rich basaltic glass occurring in fractures, and basaltic glass associated with incipient melting of the lherzolites. L_U represents glass compositions from both carbonate-bearing and carbonate-free droplets of ultramafic glass. Two-liquid fields occurring in the system leucite-fayalite- SiO_2 ¹⁰ are shown for comparison. *b*, Three-liquid field defined by coexisting basaltic, carbonatitic and volatile-rich ultramafic liquids from two representative samples. Abbreviations as for (*a*); L_C - L_U tie-lines omitted. Bulk composition of carbonate-bearing L_U was estimated by point counting. Also shown are compositional fields for ultramafic lamproites from Western Australia (L) and kimberlites (K). *c*, Effect of fractional crystallization of amphibole from three-liquid assemblage consisting of basaltic + carbonatitic + volatile-rich ultramafic liquids. Filled circles: carbonate-free ultramafic glass occurring as inclusions in lherzolite phases adjacent to amphibole-veins; filled square: pargasitic amphibole in veins; filled diamond: most primitive host basanite. Abbreviations as for (*a*).



on the shelf, and the whole gulf becomes involved in gravity current flow.

Previous studies⁵⁻⁷ have shown that there is strongly stratified flow in the gulf mouth in winter, with saline gulf water flowing out under an inflow originating on the shelf. We describe the first survey of this outflow from the headwaters of the gulf to beyond the shelf break, carried out in the Royal Australian Navy RV *HMAS Cook*. The track of the saline outflow is perhaps best illustrated by contours of bottom salinity (Fig. 1). Within the gulf, the flow tends to follow the central channel, but shows a preference for emerging from the gulf mouth on the eastern side. It then flows across the end of Investigator Strait and down the western coast of Kangaroo Island before cascading off the shelf in the region of Du Couedic canyon. The width of the current varies between 50 km at the mouth of the gulf and 20 km at its narrowest point near Cape Borda. A longitudinal section following the current (Fig. 2) shows that it has a thickness of between 20 and 40 m, and that it finds its own density level after leaving the shelf at a depth of 250 m.

The dynamics of the current in the region of the mouth and western end of Investigator strait have been discussed in a previous publication⁷ and it is encouraging to find that the path and also the structure of the current in that region are the same in the present survey. The direction of flow can be explained by assuming that the current is influenced by gravity, Coriolis and frictional forces. A simple analysis shows that the current tends to flow across depth contours at an angle of approximately 40°. This is a considerably steeper angle than that observed for gravity currents in the deep ocean⁸, and reflects the fact that the outflow is much thinner than those flows and therefore feels friction to a greater extent. The fuller data set reported here shows the effect of friction on the current as it flows across the continental shelf. Between the gulf mouth and the shelf break, the current falls vertically by 150 metres, along a path length of approximately 150 km. The effect of friction explains, in particular, why the current flows across the mouth of Investigator Strait, and does not turn into it as a consideration of Coriolis forces alone would suggest.

In the absence of direct current meter observations, it is possible to make an indirect estimate of the speed of the current using a form of the Chezy equation⁹

$$u = \left(\frac{g'h \sin \theta}{k} \right)^{1/2}$$

Here, u is the current speed, g' the reduced gravity in the flow,

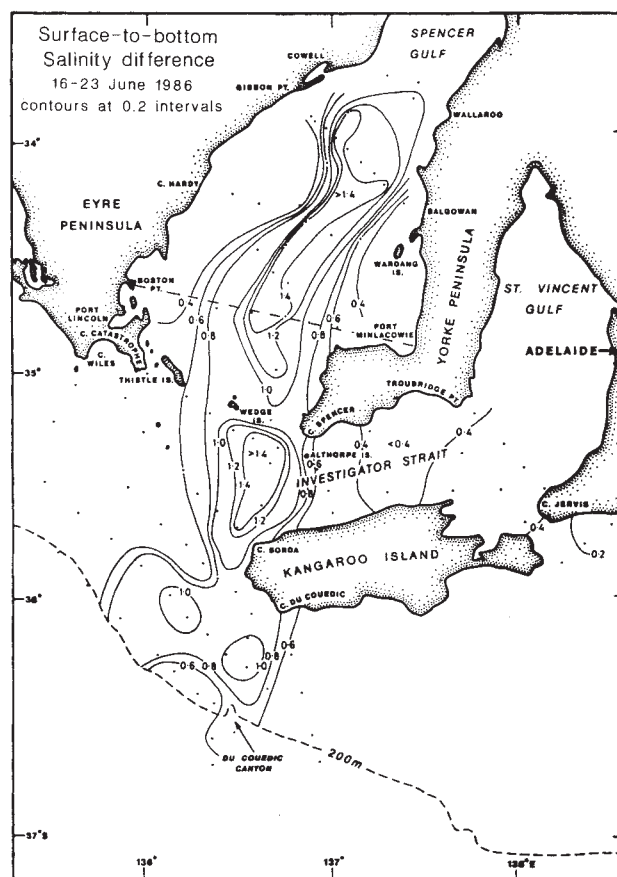


Fig. 3 Surface-to-bottom salinity difference during the survey. Off the continental shelf, the maximum salinity was taken instead of the bottom salinity. Contours are at intervals of 0.2.

θ the angle of the slope down which the current is flowing, h the thickness of the current and k a combined bottom and interfacial friction coefficient. From the section shown in Fig. 2, the maximum bottom slope is of the order of 10^{-3} . Using $g' = 10^{-2}$, $h = 20$ m and $k = 6 \times 10^{-3}$, we therefore obtain an order of magnitude estimate of the flow speed of 0.1 m s^{-1} .

This estimate can be used to calculate the probable duration of the flow. Considerations of water and salt continuity lead to an equation for the volume flux of the outflow of the form

$$O = \frac{AES}{\Delta S}$$

where A is the surface area of the Gulf, E the nett annual evaporative loss, S the salinity of the inflow, and $S + \Delta S$ the salinity of the outflow. Using appropriate values for Spencer Gulf of $A = 24 \times 10^9 \text{ m}^2$, $E = 1.25$ metres, $\Delta S = 1$ and $S = 36$, we obtain $O = 10^{12} \text{ m}^3 \text{ yr}^{-1}$. The cross-sectional area of the outflow near the mouth is approximately 10^6 m^2 , and assuming a mean flow rate of 0.1 m s^{-1} at that section, the outflow would have to continue for approximately three months to remove the salt that accumulates in the gulf during summer evaporation.

The results presented here have shown the scale of the contribution that gravity currents make to the exchange of water between Spencer Gulf and the shelf, both in the physical extent of the outflow and its persistence in time. The same mechanisms of gravitational instability and the formation of gravity-driven flows apply equally to other natural situations, for example in the production of cold salty water in polar regions contributing to the character of the deep ocean water masses. A striking new

finding of the present survey is the evidence for variability in the current, which is most clearly shown by the changing levels of stratification (Fig. 3). The constriction in the current to the west of Cape Borda and its subsequent widening also suggest that the flow is not constant. The reasons for this are open to speculation at this stage. One possibility is that the flow is modulated by the strong spring-neap tidal cycle in the gulf. If the current speed is of the order of 0.1 m s^{-1} , a fortnightly modulation would produce pulses 150 km apart. This is consistent with the distance between the two maxima in stratification shown in Fig. 3.

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1. Simpson, J. E. *A. Rev. Fluid Mech.* **14**, 213–234 (1982).
2. Griffiths, R. W. *A. Rev. Fluid Mech.* **18**, 59–89 (1986).
3. Nunes, R. A. & Lennon, G. W. *Aust. J. Marine and Freshwater Sci.* **37**, 39–53 (1986).
4. Nunes, R. A. & Lennon, G. W. *J. geophys. Res.* (in the press).
5. Bullock, D. A. *Trans. R. Soc. S. Aust.* **99**, 43–57 (1975).
6. Bye, J. A. T. & Whitehead, J. A. Jr *Estuarine coastal Mar. Sci.* **3**, 477–481 (1975).
7. Bowers, D. G. & Lennon, G. W. *Continental Shelf Res.* (in the press).
8. Bowden, K. F. *Tellus* **12**, 418–426 (1960).
9. Komar, P. in *The Sea* Vol 6 (Wiley Interscience, New York, 1977).

Benzthiazoles in estuarine sediments as indicators of street runoff

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Street runoff can be a major source of potentially toxic aromatic compounds that enter estuaries, embayments and ocean inlets^{1,2}. While investigating contaminated sediments in San Francisco Bay we discovered several benzthiazoles, which appear to be derived from the use of anti-oxidants in the manufacture of rubber tyres. Our investigations confirm that two of these compounds, benzthiazole and 2-(4-morpholinyl)-benzthiazole, occur in street runoff and that they can result from the weathering of a commercially used anti-oxidant in rubber manufacture. These compounds are proposed as potential indicators of the contribution of street runoff to the contaminants in sediments of urban coastal areas.

It is often difficult to differentiate and to quantify the magnitude and major sources of polynuclear aromatic hydrocarbons (PAH) and other neutral aromatic compounds that accumulate in contaminated coastal sediments. Typically, chemical analyses of sediments can help find the sources of aromatic hydrocarbon contaminants (for example, fossil fuel combustion products) in the environment by identifying the degree and type of polyaromatic alkylation or by determining the ratios of various aromatic and aliphatic hydrocarbons^{1,3–5}. However, before deposition in marine sediments, these contaminant indicators may be affected significantly by differential chemical behaviour, microbial metabolism, and photodegradation. Another way of estimating the contribution of PAH to coastal sediments from urban runoff is to use patterns of precipitation and road usage to calculate loading factors⁶. Obviously, the existence of marker compounds to identify clearly street runoff would greatly aid in the assessment of the proportion of estuarine contaminants derived from this chronic source.

Benzthiazole compounds are either by-products of synthesis, or degradation products of substances used as antioxidants and curing compounds in rubber manufacturing. In particular, these curing compounds are used extensively in the manufacture of

Table 1 Concentrations of 2-(4-morpholinyl)-benzthiazole in environmental samples

Sample type	Concentration ($\mu\text{g kg}^{-1}$, dry wt, $\pm$ s.d.)
Soil (edge of Highway 80 Berkeley)	273
San Francisco Bay (muddy sands)	
San Pablo Bay	49 $\pm$ 34
Berkeley	82 $\pm$ 32
Oakland*	360 $\pm$ 208
San Francisco Bay (shelly sand)	
Alameda	23
Abbots Lagoon (silt and sand)	ND

* This sample site is within 400 m of the east end of the San Francisco–Oakland Bay Bridge.

ND, not detectable.

automobile and truck tyres. By way of example the total production of benzthiazole derivatives in the United States in 1981 was $16.3 \times 10^7 \text{ kg}$ (ref. 7).

Because the initial chemical analyses of San Francisco Bay sediments revealed a morpholine-substituted benzthiazole (Fig. 1a, peak 2), we focused our studies on a major commercial product used in tyre manufacturing, Delac MOR. The major component of Delac MOR is 2-(morpholinothio)-benzthiazole (Fig. 2, compound I). Delac MOR was subjected to weathering (a 1 litre saturated aqueous solution was exposed to direct sunlight for 4 months). After studying the weathered sample, we identified a major compound, benzthiazole (Fig. 2, compound II) that was only a very minor component in the unweathered Delac MOR (Fig. 3). Analyses of Delac MOR by gas chromatography/mass spectrometry (GC/MS) before and after weathering revealed that 2-(4-morpholinyl)-benzthiazole and 2-methylmercapto-benzthiazole (Fig. 2, compounds III and IV respectively) are both impurities in Delac MOR that appear to be environmentally stable.

We have not identified the major component of Delac MOR (Fig. 2c) in San Francisco Bay sediments using GC/MS analysis probably because it is degraded in the environment; however we have detected compounds II, III and IV in these sediments. The mass spectra of the compounds in our samples are identical in all respects to those of the Delac MOR samples exposed to sunlight for 4 months (see for example, Fig. 1).

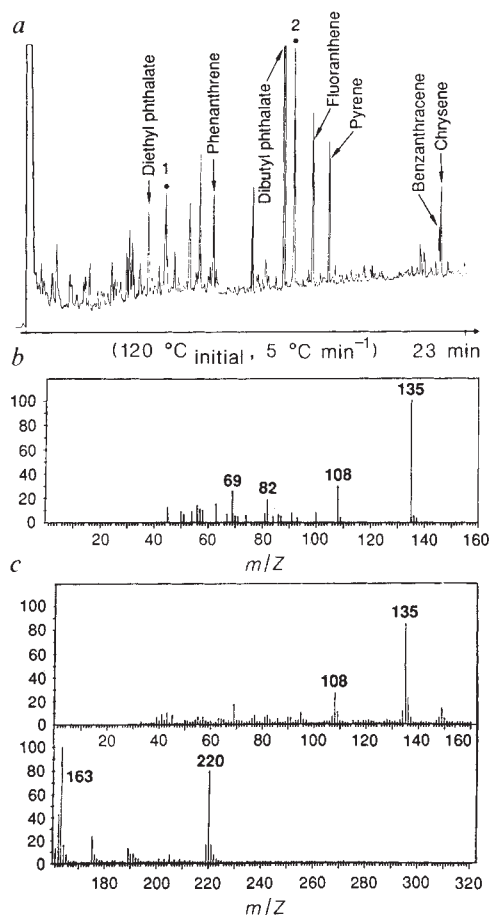


Fig. 1 Analysis of San Francisco Bay sediment sample by GC and GC/MS. A 10-g sample from Oakland Outer Harbor was dried at 60 °C and then soxhlet-extracted for 24 h. The extract was cleaned up by passing through $-C_{18}$ and $-NH_2$ reverse-phase disposable columns. Delac MOR was dissolved in UV-grade methylene chloride and analysed directly. Chromatographic separation was accomplished on a 30-m fused-silica capillary (0.25 mm, inner diameter) column (DB-1); initial temperature, 130 °C, for 5 min, followed by an increase of 5 °C min^{-1} ; detection by flame ionization or mass spectrometry. A Hewlett-Packard 5985 mass spectrometer was used in the electron impact ionization mode. *a*, Gas chromatogram of San Francisco Bay sediment sample with the peaks indicated that contain the new compounds (1, 2). Peak 1 is benzthiazole; peak 2, 2-(4-morpholinyl)-benzthiazole. *b*, Mass spectrum of peak 1. *c*, Mass spectrum of peak 2.

We synthesized III as a standard for mass spectrometry, combining aniline with carbon disulphide to produce a thiocarbonyl. This product was combined with morpholine to yield phenylthiourea which with heating produced 2-(4-morpholinyl)-benzthiazole (compound III). This standard had the same mass spectral and gas chromatographic characteristics as the substituted benzthiazole which was isolated from San Francisco Bay (Fig. 1c).

It appears therefore that a degradation product (II) and two impurities (III and IV) of Delac MOR persist in the marine environment. We did not account for microbial activity in our weathering experiment so we cannot discount the possibility that microbial metabolism is involved in the degradation of I. However benzthiazoles are known to photodecompose to stable intermediates and photodegradation appears to be the most likely mode of degradation⁸.

Quantitative analyses of sediment and soil for III, revealed high concentrations in soil samples taken within 1 m of a major highway that borders the eastern shore of central San Francisco

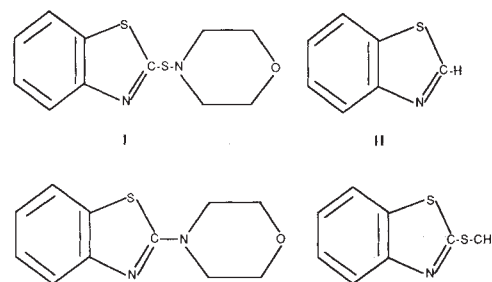


Fig. 2 Benzthiazoles; I, Delac MOR, 2-(morpholinothio)-benzthiazole [m/z 252 (M^+ , 7%), 167 (95%), 86 (100%), 108 (20%)]; II, benzthiazole [m/z 135 (M^+ , 100%), 108 (48%), 69 (50%)]; III, 2-(4-morpholinyl)-benzthiazole [m/z 220 (M^+ , 80%), 163 (87%), 135 (100%), 108 (45%)]; and IV, 2-methylmercapto-benzthiazole [m/z 181 (M^+ , 100%), 148 (90%), 108 (61%)].

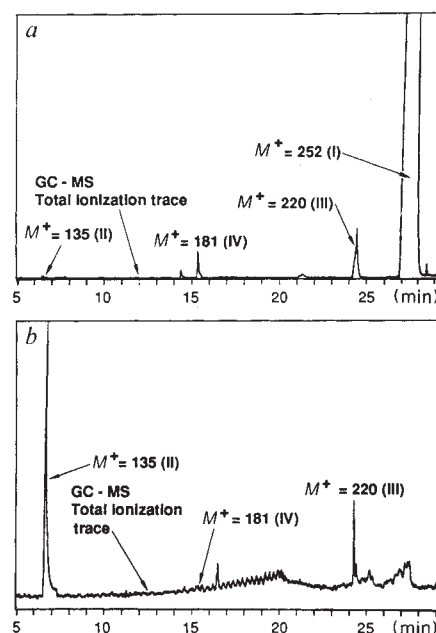


Fig. 3 *a*, Total ionization plot of Delac MOR sample, showing presence of small amounts of impurities (M^+ = 220, 181, 135). *b*, Total ionization plot of a weathered Delac MOR sample, showing that a compound with m/z = 220 remains and a large amount of a compound with m/z = 135 (II) has appeared.

Bay and in sediments adjacent to more urban areas of the Bay (Fig. 4, Table 1). The low value for the Alameda site, which is adjacent to a heavily urbanized area, is probably due to the low affinity of these compounds for the coarse sand at this site. Qualitative mass spectral analyses of extracts of a 160-ml sample of street runoff taken in Livermore, California, 3 hours after the start of the first significant rain of the summer revealed the presence of compounds II and III. Extracts of liver of starry flounder (*Platichthys stellatus*) collected from the Berkeley area also contained detectable quantities of II and IV when analysed qualitatively by GC/MS using ion monitoring techniques (m/z 181, 148 and 108), indicating that these compounds may also bioaccumulate in the estuarine food web.

To explore the possibility that the benzthiazoles that we have detected might have been contributed to sediments from natural sources, we analysed a large sediment sample from Abbots Lagoon, a pristine freshwater lagoon surrounded by native vegetation in the Point Reyes National Seashore in northern California. The nearest road to the lagoon has little traffic and is more

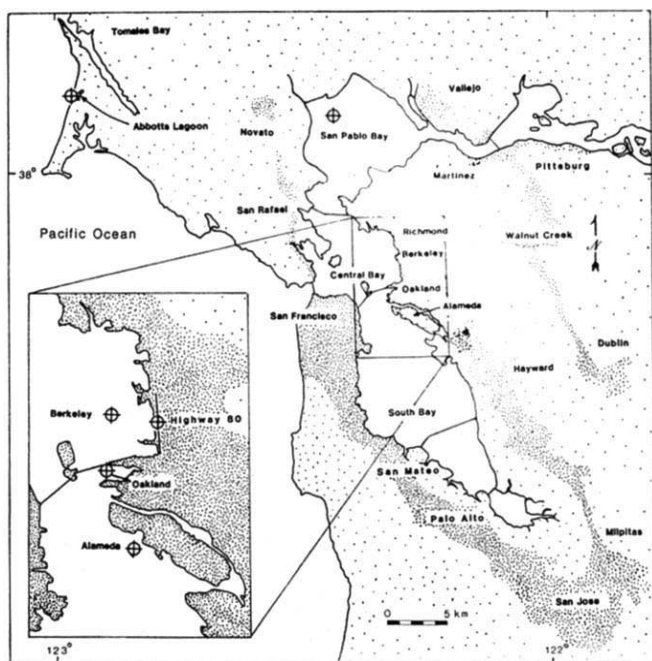


Fig. 4 Location of sampling stations in northern California. Collection sites are indicated by circles with crosses. Densely populated areas within the catchment area of San Francisco Bay are indicated by heavy stippling.

than 1,500 m from our sampling location. Our analysis of this sample by GC/MS did not detect any of the benzthiazoles in Fig. 2. (The detection limits for benzthiazoles were in the range of 5–10 ng g⁻¹.)

Both II and IV have been identified as persistent contaminants in the waste water from tyre manufacturing⁹; therefore it may not be possible to use these two compounds as tracers for street runoff when they go into estuaries that also receive large quantities of wastes from tyre manufacturing. There are no large tyre-manufacturing plants in the San Francisco Bay area. Also, the occasions when street dust is suspended in the atmosphere and blown into coastal waters will be an additional entry route for these compounds.

Little is known of the toxicity, mutagenicity or carcinogenicity of the benzthiazoles and related compounds reported here. Two compounds, component II and 4-morpholinyl-2-benzthiazole disulphide, had negative assays for mutagenicity in the Ames test¹⁰. One brief report indicated that I is mutagenic and capable of cell transformation in animal tests¹¹; but as this compound appears to be photolabile it may not affect estuarine organisms. Some preliminary experiments with IV, where 200 mg kg⁻¹ was injected intraperitoneally into rats, gave no evidence of maternal toxicity, fetal toxicity or teratogenesis¹². Because so little is known about the biological effects of benzthiazoles isolated from sediments of San Francisco Bay, further studies of these compounds are needed to determine whether they have any effect on estuarine organisms.

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1. Hamilton, S. E., Bates, T. S. & Cline, J. D. *Environ. Sci. Technol.* **18**, 72–79 (1984).
2. Payne, J. F., Martins, I. & Rahimtulla, A. *Science* **200**, 329–330 (1978).
3. Hoffman, E. J., Mills, G. L., Latimer, J. S. & Quin, J. G. *Environ. Sci. Technol.* **18**, 580–587 (1984).
4. Wakeham, S. G. thesis Univ. Washington, Seattle (1976).
5. Boehm, P. D. & Farrington, J. W. *Environ. Sci. Technol.* **18**, 840–845 (1984).
6. Hoffman, E. J., Latimer, J. S., Hunt, C. D., Mills, G. L. & Quinn, J. G. *Wat. Air Soil Pollut.* **25**, 349–364 (1985).
7. United States International Trade Commission *Synthetic Organic Chemicals, United States Production and Sales 1981* USITC Publication 1292 (US Government Printing Office, Washington DC, 1982).
8. Cutforth, H. G. & Selwood, P. W. *J. Am. chem. Soc.* **70**, 278–281 (1948).
9. Junglaus, G. A., Games, L. M. & Hites, R. A. *Analyt. Chem.* **48**, 1894–1896 (1984).
10. Crebelli, R., Falcone, E., Aquilina, G. & Carere, A. *Tox. Lett.* **23**, 307–313 (1984).
11. *Elastomeric News Log* 71, June (1980).
12. Hardin, B. D. et al. *Scand. J. Work. environ. Health* **7**, 66–75 (1981).

Chronostratigraphic markers in the end-Precambrian carbon isotope record of the Lesser Himalaya

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Early investigators^{1,2} noticed that the isotope age curve for sedimentary carbon contains a negative displacement in $\delta^{13}\text{C}$ at the Precambrian/Cambrian (PC/C) boundary, about 570 million years ago³, which may have accompanied the profound biotic changes⁴ from non-skeletal organisms to those with mineralized skeletons. Whereas the certainty of the $\delta^{13}\text{C}$ shift across the elusive boundary has recently been confirmed from a number of localities^{5–8}, its interpretation remains largely controversial. It has been attributed to variations in oceanic biomass fertility and concomitant fluxes of organic matter burial^{6–8}, changing rates of ocean ventilation⁹, onset of biomineralization¹⁰ and extraterrestrial causes⁵. Here we report the results of a carbon isotope study from a hitherto unexplored section of marine sediments in the Lesser Himalaya, India, which documents significant isotope variations across the PC/C boundary. These results also provide important details towards resolving the relative timing of the $\delta^{13}\text{C}$ shifts and the biotic changes. Evaluation of our data rules out post-depositional isotope exchange as a major cause of the recorded $\delta^{13}\text{C}$ variations and favours changes in the isotope chemistry of the ocean surface at the close of the Precambrian.

The Krol-Tal sedimentary succession forms large synclinal structures along the southern margin of the Himalayan belt (Fig. 1a), reaching a maximum thickness of about 3,000 m. The chronostratigraphy of this largely non-fossiliferous series (Fig. 1b) has long been a subject of controversy among geologists¹². Recently, a late Precambrian to early Cambrian age has been firmly established¹³ on the basis of: (1) identification of stromatolites of late Precambrian (Vendian stage) affinity (genera *Conophyton*, *Tungussia* and *Potomia* for example) in the Krol D Member; (2) discovery in the uppermost Krol E of skeletalized benthic fauna chiefly composed of archaeocyathid communities as well as of calcareous algal bioherms (genera *Epiphyton*, *Renalcis*) believed to have evolved exclusively at the PC/C boundary^{4,14}; (3) documentation of shelly phosphatic microfauna (such as hyolithids and protoconodonts) of unequivocal earliest Cambrian (Tommotion stage) affinity in the lower Chert Member of the Tal Formation, and (4) a record of the early Cambrian brachiopods and trilobites from the Arenaceous Member overlying the Chert Member.

Upper Krol units (Fig. 1b) consist of planar stromatolites with repetitive interbeds of sedimentary breccia and grainstone deposited on broad tidal flats. They are dolomitized on a regional scale. The topmost section of Krol E grades conformably into the Tal Formation, the lower member of which contains black

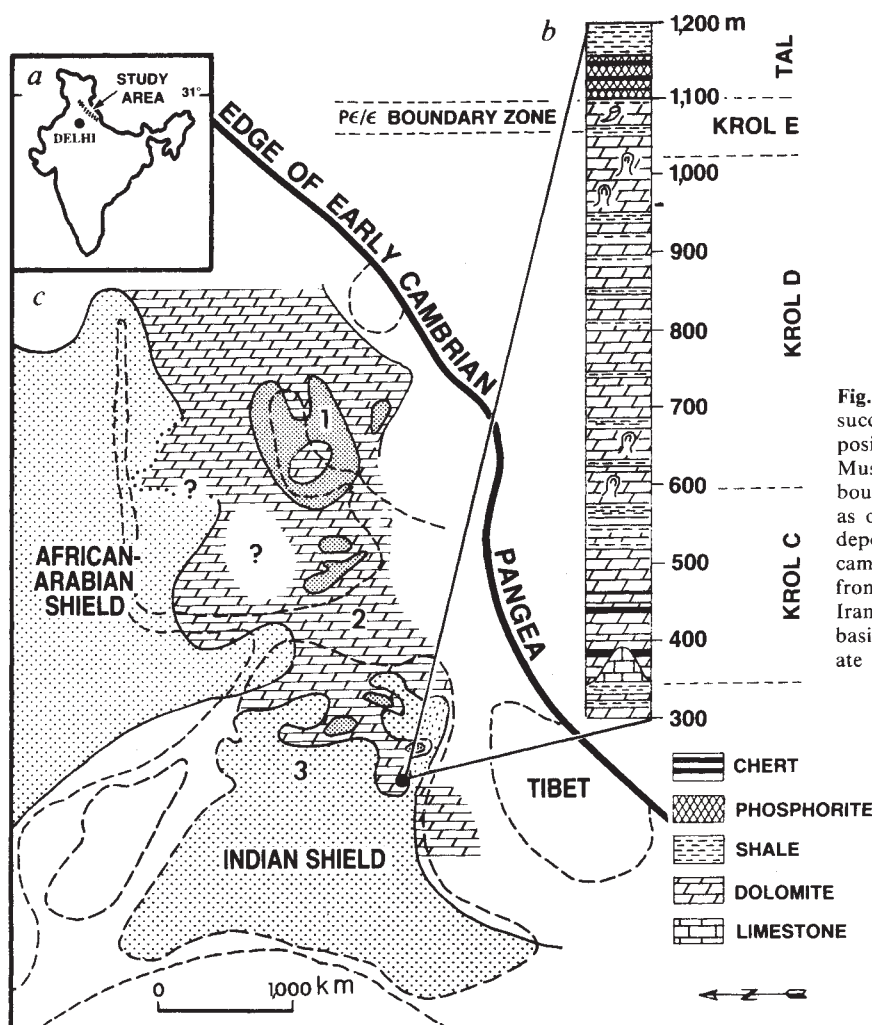


Fig. 1 Geological frame of the Krol-Tal sedimentary succession. *a*, Location map of the study area. *b*, Composite section constructed from outcrops in the Mussoorie syncline (30°29' N, 78°08' E). Krol C lower boundary is about 350 m above the Blaini tillites taken as our benchmark. *c*, Reconstruction of the Krol-Tal depositional environment in the context of the late Precambrian-early Cambrian Tethys seaway (modified from ref. 11). Thick salt deposits (Hormuz salt build-up, Iran; Salt Range, Pakistan) that formed in restricted basins (1) were interspersed between extended carbonate platforms (2) stretched along the Pangea landmass (3).

shales, chert and phosphorite with subordinate pyrite and calcite. Following this basal phase of chemical sedimentation, the Tal Formation grades upward into terrigenous clastic sediments.

If the advent of the fauna with mineralized skeletons heralds the beginning of the Cambrian^{4,14}, then the PC/C boundary should be located somewhere in the upper Krol E below the first occurrence of the archaeocyathid reefs (Fig. 1*b*). Therefore the Krol-Tal sequence, where the PC/C transition occurs within a sequence of marine carbonates of similar facies, should hold a key for the study of chemical changes^{15,16} that took place in the marine environment at the close of the Precambrian. These changes left their mark on the carbon isotope composition of the upper Krol-lower Tal sediments as shown in Figs 2 and 3.

The upper Krol organic-poor carbonates ($C_{org}=0.02$ to 0.03%) yield $\delta^{13}C$ values that are compatible with a marine carbon source²⁰ (-1.3 to 4.9%). By contrast, the organic-rich ($C_{org}=0.4$ to 2.2%)¹⁸ apatite-bound carbonate and the calcites from the Tal Chert Member display anomalously negative $\delta^{13}C$ compositions, ranging from -6.5 to -9.9% and -6.3 to -17.8% , respectively (Fig. 2). These pronounced ^{13}C -depletions bear the mark of pore waters whose carbon chemistry was greatly modified by bacterial processes operating in oxygen-deficient sediments^{21,22}. The $\delta^{13}C$ record in Fig. 3, based mainly on petrographically identifiable early fine-grained dolomites, exhibits two subordinate phases of ^{13}C -enrichment (3.3 and 3.6%) in the upper Krol C and lower Krol D respectively, followed by a major positive excursion of 7% relative to an intercalated minimum in the upper Krol D. We stress that this maximum, and its subsequent decline in the archaeocyathid reef

zone of the uppermost Krol E to about 0.3%, occurs within the same stromatolitic dolomite facies and clearly precedes the distinct facies change at the Krol-Tal contact.

With carbonates of Precambrian antiquity, the preservation of pristine isotope compositions may be questioned²³ because meteoric water diagenesis, deep burial and metamorphism had ample opportunity to alter the original isotope ratios. There is, however, convincing evidence that the Krol dolomite succession did not undergo excessive burial and concomitant deformation and, accordingly, has never gone through a major isotope re-equilibration event. First, the preserved primary fabrics of the original sediments and early cements (for example, planar stromatolites, fenestrae, ooids, calcareous algae and archaeocyathid cups) suggest that dolomitization of the Krol carbonate series was an early event²⁴. In this case early dolomites either inherit the $\delta^{13}C$ composition of their marine carbonate precursor^{24,25} or, if the fluid flow system delivering magnesium is sufficiently fast (if there is a high fluid/rock ratio), it might directly represent the $\delta^{13}C$ composition of the overlying sea water²⁶. A case in point is offered by the $^{87}Sr/^{86}Sr$ ratios of one limestone and two dolomites representing the Krol C, Krol D and uppermost Krol E lithounits which yield values of 0.70925, 0.70913 and 0.70985 respectively, in agreement with the accepted $^{87}Sr/^{86}Sr$ composition of the contemporaneous sea water²⁷.

Second, distinct textural components from the same parental rocks (Table 1) have yielded $\delta^{18}O$ and $\delta^{13}C$ compositions which, in general, show consistent differences between early dolomitic and late void-filling dolospar cements (average 7.9‰ and 2.4‰ in $\delta^{18}O$ and $\delta^{13}C$ respectively). These isotope heterogeneities of coexisting dolomite phases would argue convincingly against

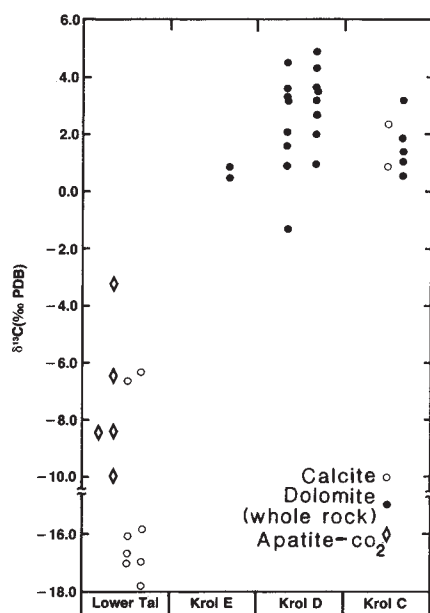


Fig. 2 Distribution of $\delta^{13}\text{C}$ in the upper Krol dolomites and calcite-apatite mineral pairs from the lower Tal Chert Member. Note that anomalously ^{13}C -depleted values occur where the chert-phosphorite association is pronounced. Dolomites were analysed for carbon and oxygen isotopes using standard procedures¹⁷. Traces of calcite (0.4 to 1.5%), contained by most dolomite samples, were removed by EDTA solution before the CO_2 extraction. Apatite-bound carbonate and calcites were analysed during a previous investigation¹⁸. $^{13}\text{C}/^{12}\text{C}$ isotope ratios are reported in the standard $\delta^{13}\text{C}$ (‰ PDB) notation¹⁹. The overall error (1σ), based on standards analyses and repeats is estimated to be $\pm 0.2\%$.

any major offset^{24,28} resulting from repartition of isotopes under a new set of boundary conditions during the postdepositional history of their parent rock. The relative proximity of spar $\delta^{13}\text{C}$ values to those of the 'pristine' microdolomite and whole rock in many of the samples analysed (Table 1) also suggests that only a negligible amount of 'external' carbon was added during the late diagenesis (the 'spar phase'), and there is no evidence of soil CO_2 contribution which normally shifts $\delta^{13}\text{C}$ to more negative values²⁹.

Carbonate cements that yield highly positive $\delta^{13}\text{C}$ values must be subjected to special scrutiny because of demonstrable links between extreme ^{13}C -fractionations and bacterial methanogenesis²¹. These complications, however, are normally restricted to organic-rich sediments having a carbonate-carbon to organic carbon ratio of less than 7 to 1 (ref. 30). The paucity of organic carbon and the dominance of carbonate in the Krol sediments ($C_{\text{carb}}/C_{\text{org}} > 430$, Table 1) therefore adds weight to our conclusion that post-depositional processes were unlikely to impair the quality of the $\delta^{13}\text{C}$ record depicted in Fig. 3.

The observed $\delta^{13}\text{C}$ shift of 7‰ in the uppermost Vendian, and its subsequent decline toward the PC/C boundary at a rate of about $0.06\% \text{ m}^{-1}$ (Fig. 3), clearly represents an excursion from normal marine isotope abundances that requires an explanation. Changes in $^{13}\text{C}/^{12}\text{C}$ of shallow marine carbonates may be due to two different causes, namely (1) global changes in the oceanic carbon^{1,2}, and (2) corresponding variations in a limited (semi-restricted) carbon pool^{31,32} in response to local conditions. The recognition of global excursions requires identification of synchronous $\delta^{13}\text{C}$ shifts in contemporaneous $\delta^{13}\text{C}$ records of both shallow and deep ocean carbon pools³³ and/or contemporaneous shallow marine carbonates of wide geographic distribution³⁴. Failure to find such shifts would indicate that observed $\delta^{13}\text{C}$ anomalies reflect local basinal rather than global events, such as the mid-Precambrian Lomagundi $\delta^{13}\text{C}$ event³².

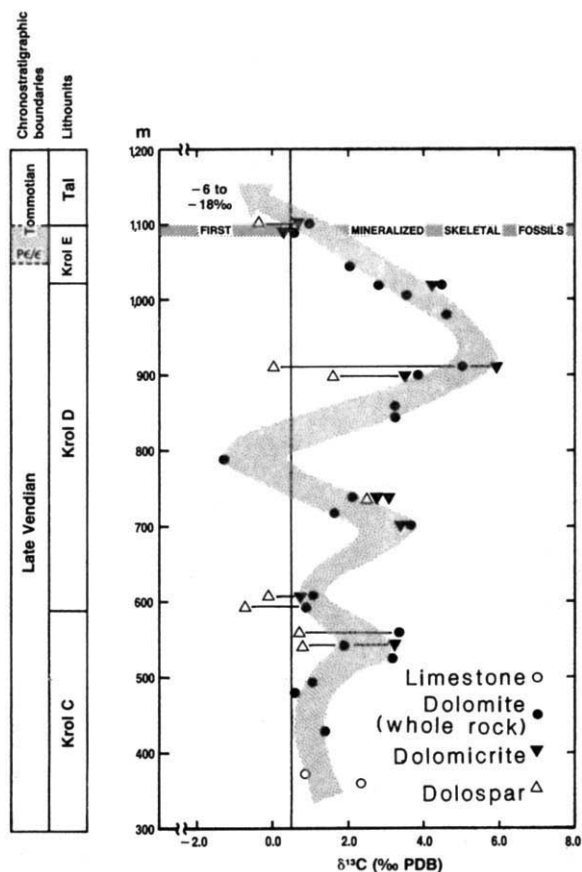


Fig. 3 $\delta^{13}\text{C}$ record of upper Krol carbonates. Coexisting early (dolomicrite) and late (dolospar) cements were separated using a fine dental drill guided by a petrographic microscope. The stippled pattern is constrained by the isotopic composition of early cements and homogeneous micritic dolomite which best represent marine $\delta^{13}\text{C}$ values (see text).

Whereas no deep-sea carbonates coeval with the Krol succession are known, comparison of the $\delta^{13}\text{C}$ record from the Lesser Himalaya with time-equivalent records from the Siberian Platform and Anti-Atlas Mountains tends to confirm the existence of a positive $\delta^{13}\text{C}$ excursion at the end of the Precambrian (Fig. 4). In all three sequences the $\delta^{13}\text{C}$ shift occurs just before the first recorded appearance of skeletalized fauna, archaeocyathids on the Siberian Platform⁷ and Lesser Himalaya¹³ and trilobites in the Anti-Atlas⁶. Although the age curves may differ in detail, there is also good agreement on the magnitude of the $\delta^{13}\text{C}$ shift between the Siberian and Himalayan records. Accordingly, time-bound global excursions of the $\delta^{13}\text{C}$ have considerable potential as precise chronostratigraphic tools which are independent of the controversial biostratigraphic markers currently used to delineate the PC/C boundary³⁵. Isotope stratigraphic markers should, however, be used with great caution because of the potential complications arising from diagenetic overprints, particularly in boundary sections where organic-rich and organic-poor carbonates alternate (for instance the Sinian System in the Yangtze Gorge sections)⁸.

On a timescale that exceeds the turnover time of carbon in the exogenic cycle ($\sim 10^5$ yr), the $\delta^{13}\text{C}$ composition of oceanic carbon is a function of the carbon fluxes into and from the carbonate (C_{carb}) and organic matter (C_{org}) pools¹. Shifts of carbon between these two major pools would inevitably affect the marine $\delta^{13}\text{C}$ balance because of the pronounced ^{13}C deficiency of organic matter ($\delta^{13}\text{C}_{\text{org}} = -25$ to -30%)^{1,32}. As the long-term steady-state ratio of the fluxes is about $C_{\text{org}}/C_{\text{carb}} = 1/4$ (Ronov ratio)¹, it follows that the 7‰ positive shift observed

Table 1 Carbon and oxygen isotope compositions of textural components in dolomites representative of the Krol lithounits

Level* (m)	Code	Lithounit	Fabric†	Dolomite‡ (%)	C _{org} § (%)	Whole Rock (% PDB)	Dolomicrite (% PDB)	Dolospar (% PDB)	(m/m + s) × 10 ² ¶ (%)
1,100	8T	Krol E	aa	99.2	0.03	0.9; -10.9	0.7; -10.8	-0.3; -13.2	44
1,090	13T		m	78.0	0.02	0.5; -2.7	0.3; -3.9	—	>95
1,025	1.2T	Krol D	sf	98.7	0.03	4.4; -1.5	4.3; -1.0	—	92
920	1.5T		sf	97.9	0.03	4.9; -4.6	5.7; -2.8	0.0; -8.6	52
900	1.4T		sf	99.5	0.03	3.6; -1.4	3.5; -0.7	1.7; -10.1	78
790	2.2T		al	95.6	0.02	-1.3; -1.8	—	—	>95
740	2.3T		al	99.0	0.02	2.1; -9.5	3.0; -2.4	2.2; -7.4	41
700	2.4T		al	99.0	0.03	3.6; -1.6	3.4; -1.4	—	87
610	2.11T		ab	99.3	0.02	1.0; -8.2	0.8; -8.2	0.0; -14.6	45
590	2.12T		ab	99.1	0.03	0.9; -9.8	—	-0.7; -14.1	34
560	2.31T	Krol C	al	98.8	0.02	3.4; -4.3	—	0.6; -10.5	58
540	4T		z	9.1	0.02	1.9; -12.0	3.3; -1.7	0.8; -11.1	28

* Stratigraphic position as indicated in Fig. 3.

† Symbols: aa, algal-archaeocyathid dolomite; m, mosaic dolomite replacing limemud; sf, planar stromatolite with fenestrae; al, algal mat dolomite; ab, algal mat breccia; z, 'zebra' dolomite.

‡ X-ray diffraction data.

§ Total organic carbon values were determined with a Rock Eval II unit having a reproducibility of $\pm 5\%$ of value.

|| Left column, $\delta^{13}\text{C}$ values; right column $\delta^{18}\text{O}$ values. Dolomicrite, early microcrystalline dolomite; Dolospar, coarse void filling late dolomite.

¶ Ratios of early (m) to late (s) dolomites were derived from petrography. Estimates are assigned an error of $\pm 10\%$.

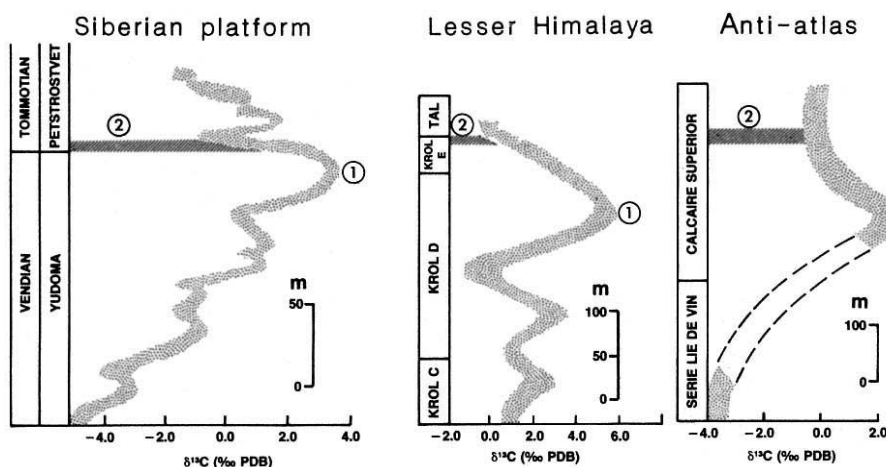


Fig. 4 Correlation between three time-equivalent sections (Siberian Platform⁷, Lesser Himalaya and Anti-Atlas⁶) of comparable resolution using the $\delta^{13}\text{C}$ positive excursions (1) and the first appearance of skeletalized fauna (2) as fixed points.

in the late Vendian (Fig. 3) could be attributed to a change of the Ronov ratio to 1/2.3. A worldwide phase of phosphogenesis¹⁶ at, or near, the PC/C boundary supports the inference⁶⁻⁸ of biological controls driven by ocean fertility changes and acting on the marine carbon reservoir.

The alternative, that the $\delta^{13}\text{C}$ excursions and the lithological changes across the Krol-Tal Formations reported here may constitute different expressions of ocean ventilation changes^{9,15} at the PC/C boundary, remains, however, an intriguing possibility which should be addressed by future studies.

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- Schidlowski, M., Eichmann, R. & Junge, C. E. *Precambrian Res.* **2**, 1-69 (1975).
- Veizer, J. & Hoefs, J. *Geochim. cosmochim. Acta* **40**, 1387-1395 (1978).
- James, H. L. *Precambrian Res.* **15**, 191-198 (1981).
- Stanley, S. M. *Am. J. Sci.* **276**, 56-76 (1976).
- Hsu, K. J. *et al. Nature* **316**, 809-811 (1985).
- Tucker, M. E. *Nature* **319**, 48-50 (1986).

- Magaritz, M., Holser, W. T. & Kirschvink, J. L. *Nature* **320**, 258-259 (1986).
- Lambert, I. B., Walter, M. R., Wenlong, Z., Songnian, L. & Guogan, M. *Nature* **325**, 140-142 (1987).
- Goodfellow, W. D. *Nature* **322**, 116-117 (1986).
- Degens, E. T., Kazmierczak, J. & Ittekkot, V. *Tschermaks miner. petrogr. Mitt.* **35**, 117-126 (1986).
- Gorin, G. E., Racz, L. G. & Walter, M. R. *Am. Ass. Petrol. Geol. Bull.* **66**, 2609-2627 (1982).
- Banerjee, D. M. *Geol. Surv. Bull. Finl.* **331**, 73-89 (1985).
- Singh, I. B. & Rai, V. *J. Paleont. Soc. India* **28**, 67-90 (1983).
- Rozanov, A. Yu. *Episodes* **7**, 20-24 (1984).
- Holser, W. T. *Nature* **267**, 403-408 (1977).
- Cook, P. J. & Shergold, J. H. *Nature* **308**, 231-236 (1984).
- McCrea, J. M. *J. chem. Phys.* **18**, 849-857 (1950).
- Banerjee, D. M., Schidlowski, M. & Arneith, J. D. *Precambrian Res.* **33**, 239-253 (1986).
- Craig, H. *Geochim. cosmochim. Acta* **12**, 133-149 (1957).
- Clayton, R. M. & Degens, E. T. *Am. Ass. Petrol. Geol. Bull.* **43**, 890-897 (1959).
- Irwin, H., Curtis, C. & Coleman, M. *Nature* **269**, 209-213 (1977).
- Kolodny, Y. *Israel J. Earth Sci.* **29**, 147-156 (1980).
- Veizer, J. *J. sedim. Petrol.* **47**, 565-581 (1977).
- Tucker, M. E. *Geology* **10**, 7-12 (1982).
- Aharon, P., Kolodny, Y. & Sass, E. *J. Geol.* **85**, 27-48 (1977).
- Aharon, P., Socki, R. A. & Chan, L. *J. Geol.* **95**, 187-203 (1987).
- Veizer, J., Compston, W., Clauer, N. & Schidlowski, M. *Geochim. cosmochim. Acta* **47**, 295-302 (1983).
- Fritz, P. *Earth planet. Sci. Lett.* **11**, 277-282 (1971).
- Hudson, J. D. *J. geol. Soc. Lond.* **133**, 637-660 (1977).
- Scholle, P. A. & Arthur, M. A. *Am. Ass. Petrol. Geol. Bull.* **64**, 67-87 (1980).
- Aharon, P. in *The Carbon Cycle and Atmospheric CO₂: Natural Variations Archaean to Present* (eds Sudquist, E. T. & Broecker, W. S.) 343-355 (Am. Geophys. Union, Washington DC, 1985).
- Schidlowski, M., Eichman, R. & Junge, C. E. *Geochim. cosmochim. Acta* **40**, 449-455 (1976).
- Broecker, W. S. *Geochim. cosmochim. Acta* **46**, 1689-1705 (1982).
- Magaritz, M., Anderson, R. Y., Holser, W. T., Saltzman, E. S. & Garber, J. *Earth planet. Sci. Lett.* **66**, 111-124 (1983).
- Cowie, J. W. & Rozanov, A. Yu. *Geol. Mag.* **120**(2), 129-139 (1983).

Benthic decomposition of organic matter at a deep-water site in the Panama Basin

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Deep-sea sediments are linked to processes in surface waters by the sinking of particulate matter¹⁻³. These particles represent the supply of energy for the biota of most of the deep sea⁴. The particulate matter that sinks through the water column to a deep-water site (3,856 m deep) in the Panama Basin (North Malpelo Flat; 5°21' N, 81°53' W) consists largely of coccolithophorids and associated macroaggregates⁵. The surface sediments at the same site were extremely depleted in these biogenic materials, suggesting rapid rates of mineralization in a narrow transition layer near the sediment/water interface. Here we present evidence suggesting that 50–85% of the organic carbon that reaches these deep-sea sediments is remineralized within one year. The decomposition of these recently surface-derived particles may account for essentially all of benthic decomposition at this site.

Sediment-trap experiments at our well-studied site in the Panama Basin have demonstrated that a large fraction of the biogenic particulate flux consists of coccolithophorids, *Umbilicosphaera sibogae* (an occasional dominant under bloom conditions) and *Emiliania huxleyi* (a ubiquitous species during most of the year)⁵⁻⁹. During June–July 1981, for example, *U. sibogae* alone accounted for more than 90% of the carbonate sinking at this site⁷. To test the potential of the benthic environment to cause dissolution and decomposition of these coccolithophorids, we axenically grew both *U. sibogae* and *E. huxleyi* in the laboratory¹⁰, labelled with ¹⁴C. The material was harvested by settling and stored frozen in precisely measured aliquots. On board ship, aliquots of the labelled algal suspension were added to plexiglass chambers (open tubes, 6 cm diameter by 18 mm height), the wide (open) ends of which were covered with Nitex netting of pore-size 1 µm. The large pore size permits very rapid exchange of dissolved materials (50% exchange in 2 h) but prevents the entry or escape of particles larger than 1 µm¹¹. The concentration of material within the chambers was small (30 µg l⁻¹) to avoid problems from overpacking. The specific activity of the initial material was high enough (0.13 µCi µmol⁻¹ C) to allow measurement of a 3% loss of material. The chambers, attached to minimooring arrays, were placed in protective boxes and brought to the sea floor at depth 3,900 m by the deep submergence research vessel (DSRV) *Alvin*. The minimooring arrays deployed the samples in three ways: (1) at the sediment/water interface, (2) 10 cm above the interface and (3) 1 m above the interface. As a control for losses during deployment and handling, and to correct for the solubilization caused by freezing and thawing, some chambers (four for each species) were carried to the sea floor and immediately returned to the surface. Ten to thirteen chambers from each type of deployment and species were recovered by (DSRV) *Alvin*, 7 to 11 days after deployment; the remainder (for each species: 21 at the interface; 16 at 10 cm and 7 at 1 m) were recovered one year later. Carbon content of the material was measured by the carbon analyser after extraction in 0.1 M H₃PO₄ to remove any carbonate; the ¹⁴C content was measured by liquid scintillation counting. We measured all the ¹⁴C that remained in and on each chamber and fractionated this material, after removing the carbon-

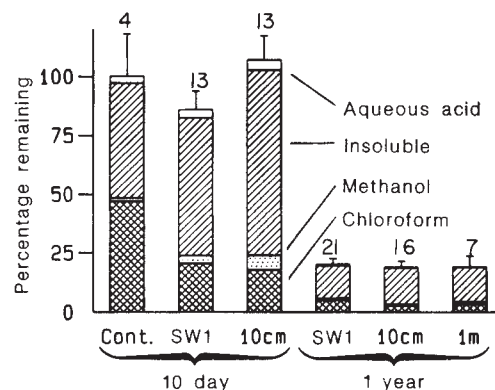


Fig. 1 Experimental decomposition of the organic fraction of the coccolithophorid, *U. sibogae* at depth 3,900 m in the Panama Basin. The coccolithophorid, labelled with ¹⁴C was placed in permeable chambers, carried to the sea floor by DSRV *Alvin* and deployed in three ways. Deployments were at the sediment/water interface 10 cm above the interface and 1 m above the interface with a minimooring array. Recoveries were 10 d and 1 yr after deployment. 'Cont.' refers to a control in which samples were brought to the sea floor but recovered immediately. The hatching represents the extractable fractions analysed¹² after removal of carbonate; standard error is shown for the total organic material. The number of chambers sampled is indicated above the bars; 100% represents 30,897 d.p.m.

ate, into several functional organic groups^{12,13}. This experiment gives an estimate of the potential of the near-benthic environment to remineralize and/or dissolve fresh surface-derived materials. At the end of one year, ~80% ± 4% standard deviation; N, the number of replicates = 44; one-year deployments combined; see Fig. 1) of organic carbon from *Umbilicosphaera* and 85% (±10% s.d., N = 42) of organic carbon from *Emiliania* had been decomposed, dissolved, or fragmented finely enough to leave the 1-µm mesh chambers (Fig. 1), compared with the control or 10-day deployments. Assuming a first-order decay model, the mean residence time of this material on the sediments would be about 0.7 y, and the apparent decay constant would be $\geq 5 \times 10^{-8} \text{ s}^{-1}$. This decay rate is roughly 100-fold greater than that estimated by Emerson *et al.*¹⁴ from profiles of organic-C concentration in the sediments at the MANOP sites at comparable depths, and nearly 1,000-fold greater than those reported by Reimers and Suess¹⁵ based on modelling of Pacific sediments of the Antarctic Ridge. Our decay rates do agree, however, with the estimated decomposition of particulates as they sink through the abyssal water column. Using the data and models of Suess¹ and of Martin *et al.*¹⁶ and a conservative assumed sinking speed of 10 m d⁻¹, we calculate apparent first-order decay rates of 4×10^{-8} – $5 \times 10^{-8} \text{ s}^{-1}$ for sinking particles below a depth of 1,000 m.

To compare our decomposition experiment with the *in situ* rates, we estimated benthic carbon remineralization with two independent methods, steady-state burial rates and pore-water oxygen profiles. Using DSRV *Alvin*, we obtained both tube cores and box cores under direct human observation such that the sediment/water interface was recovered intact. These cores were frozen and sectioned at intervals of 1–5 mm for bulk sedimentological analysis. Relative to the non-combustible lithogenic fraction (largely smectite)¹⁷ all of the biogenic constituents are extremely depleted even within the top few millimetres of the core, in comparison with the material caught by a sediment trap deployed only 50 m above the sea floor (Fig. 2). In the absence of significant, near-benthic horizontal transport at this site⁵, the observed chemical changes between the sinking particulate material and the sediment surface can be attributed to *in situ* reactions. Assuming that the non-combustible lithogenic fraction undergoes no significant post-depositional dissolution or

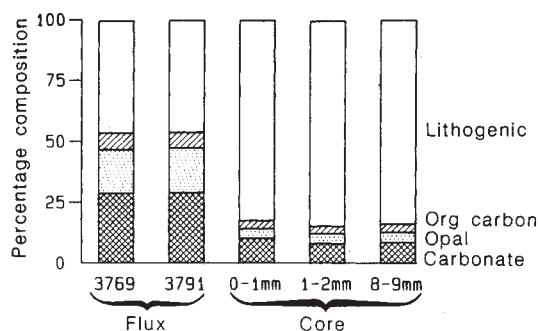


Fig. 2 Percentage composition (by mass) of particulate flux intercepted by sediment traps at 3769 and 3791 m and of the surface sediments, at three horizons, at depth 3900 m in the Panama Basin. The organic carbon content of these sediments was essentially constant at depths from 1 to 20 cm.

physical removal at this site, we can use the input and accumulation of this fraction to calculate changes in the other fractions¹⁸⁻²⁰. Using this approach, we calculate a steady-state carbon remineralization rate of $18 \mu\text{mol C cm}^{-2} \text{ yr}^{-1}$.

Also using DSRV *Alvin* to collect intact sediment cores, we made measurements of pore-water dissolved oxygen concentrations by microelectrode at depth intervals of approximately $100 \mu\text{m}$ ²¹. The tube cores were analysed on deck immediately after DSRV *Alvin* was recovered²² and oxygen consumption was calculated using the approach of Revsbech *et al.*²³, which relies on h , the depth at which the oxygen concentration becomes zero, and on the porosity of the sediments. In the six cores that we analysed, h ranged from 9.5 to 11.3 mm and averaged 10.7 mm, and porosity averaged 89%. The concentration of oxygen in the overlying water was measured in a sample collected by a go-flo bottle that was suspended from DSRV *Alvin*'s basket approximately 10 cm above the sediment/water interface. Following the equations and assumptions of Revsbech *et al.*²³ our calculated areal rate of oxygen consumption would be $17-30 \mu\text{mol O}_2 \text{ cm}^{-2} \text{ yr}^{-1}$. This oxygen consumption rate is within the published range for oxygen consumption at comparable depths measured by direct respirometry^{24,25}. Assuming a respiratory ratio of 1.2 mol O_2 consumed per mole of CO_2 produced, we arrive at an estimate of carbon remineralization of about $14-25 \mu\text{mol C cm}^{-2} \text{ yr}^{-1}$, which compares favourably with the estimate based on flux and decomposition ($18 \mu\text{mol C cm}^{-2} \text{ yr}^{-1}$).

The traditional view of the deep sea is that decomposition proceeds very slowly, and that the residence time of organic matter on the deep-sea floor is long^{26,14}. Our data and other recent evidence²⁷⁻²⁹ challenge this view. Our experimental studies indicate that fresh, surface-derived organic material, such as coccolithophorids, decompose no slower than 75-85% per year at this site. The large particulate fluxes, at this site, of chlorophyll and intact algal cells⁹, and the large seasonal variation in particulate flux^{7,9} all demonstrate that some relatively fresh surface-derived material reaches the sediments. If this fresh material were representative of roughly 70% of the organic-C input to the sediments, decomposition of this fresh material could account for essentially all of the organic-C oxidation and oxygen consumption at this deep-sea site. Although the Panama Basin site is a somewhat unique oceanographic setting in that it is a productive site over deep-water⁶, data at other sites support our view. Smith and Baldwin²⁵ reported that measured oxygen consumption in deep-sea Pacific sediments was seasonally variable and probably responded to changes in particulate flux from surface waters³⁰. Our result, that fresh, surface-derived particulate material decomposes rapidly at abyssal depths, lends support to their direct observations of benthic respiration, and suggests that the deep-sea sediment metabolism may be more

dynamic, and more closely coupled to pelagic processes, than previously thought.

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1. Suess, E. *Nature* **288**, 260-263 (1980).
2. Honjo, S. *Mar. Micropaleont.* **1**, 65-79 (1976).
3. McCave, I. N. *Deep Sea Res.* **22**, 491-502 (1975).
4. Jannasch, H. W. & Mottl, M. *Science* **229**, 717-725 (1985).
5. Honjo, S., Spencer, D. W., & Farrington, J. W. *Science* **216**, 516-518 (1980).
6. Forsberg, E. D. *Bull. inter-Am. trop. Tuna Comm* **14**, 49-258 (1969).
7. Honjo, S. *Science* **218**, 883-884 (1982).
8. Honjo, S., Manganini, S. J. & Cole, J. J. *Deep Sea Res.* **29**, 609-625 (1982).
9. Cole, J. J., Honjo, S. & Caraco, N. *Hydrobiologia* **122**, 193-197.
10. Guillard, R. L. in *Culture of Marine Invertebrate Animals* (eds Smith, W. L. & Chanley, M. H.) 29-60 (Plenum, New York, 1975).
11. Cole, J. J. in *An Ecosystem Approach to Aquatic Ecology; Mirror Lake and its Environment* (ed. Likens, G. E.) 302-310 (Springer, New York, 1980).
12. Li, W. K. W., Glover, H. E. & Morris, I. *Limnol. Oceanogr.* **25**, 447-455 (1980).
13. Cuhel, R. L., Jannasch, H. W. & Taylor, C. D. *Limnol. Oceanogr.* **28**, 1-18 (1983).
14. Emerson, S., Fischer, K., Reimers, C. & Heggie, D. *Deep Sea Res.* **32**, 1-22 (1985).
15. Reimers, C. E. & Suess, E. *Mar. Chem.* **13**, 141-168 (1983).
16. Martin, J. H., Knauer, G. A., Karl, D. M. & Broenkow, W. W. *Deep Sea Res.* (in the press).
17. Honjo, S., Manganini, S. J. & Poppe, L. J. *Mar. Geol.* **50**, 199-220 (1982).
18. Dymond, J. & Lyle, M. *Limnol. Oceanogr.* **30**, 699-712 (1985).
19. Cobler, R. & Dymond, J. *Science* **209**, 801-803 (1980).
20. Bender, M. L. & Heggie, D. T. *Geochim. cosmochim. Acta* **48**, 977-986 (1984).
21. *Cruise Report of RV Atlantis II Leg 112-3* (Woods Hole Oceanographic Institute, Massachusetts, 1984).
22. Reimers, C. E., Kalhorn, S., Emerson, S. R. & Nealson, K. H. *Geochim. cosmochim. Acta* **48**, 903-910 (1984).
23. Revsbech, N. P., Jorgenson, B. B. & Blackburn, T. H. *Science* **207**, 1355-1356 (1980).
24. Smith, K. L. *Mar. Biol.* **47**, 337-347 (1978).
25. Smith, K. L. & Baldwin, R. J. *Nature* **307**, 624-626 (1984).
26. Jannasch, H. W. *Bioscience* **29**, 228-232 (1979).
27. Gardner, W. D., Hinga, K. R. & Marra, J. J. *Mar. Res.* **41**, 195-214 (1983).
28. Cahet, G. & Sibuet, M. *Mar. Biol.* **90**, 307 (1986).
29. Rowe, G. T. & Deming, J. W. *J. mar. Res.* **43**, 925-950 (1985).
30. Deuser, W. G. & Ross, E. H. *Nature* **283**, 364-365 (1980).

A novel mechanism for regulating the excitation of photosystem II in a green alga

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Photosynthetic organisms alter their photosynthetic pigment composition in response to changes in growth irradiance¹⁻³. Photoadaptation maximizes light-harvesting when photon flux densities are low, and minimizes photo-oxidative damage to the photosynthetic machinery at high light levels. In chlorophytes, the major light-harvesting antenna is light-harvesting complex II (LHC II), a family of proteins binding chlorophyll *a* (Chl *a*), Chl *b*, and carotenoids, and accounting for 40-60% of total cell chlorophyll⁴. LHC II is associated principally with photosystem II, with reversible phosphorylation of LHC II regulating short-term adjustments in energy distribution to photosystem I⁵. Previous studies on green algae and higher plants have emphasized the longer-term adaptive importance of the inverse relationship between growth irradiance and the proportion of total cellular chlorophyll associated with LHC II⁶⁻⁸. In higher plants the pigment composition of LHC II appears to be highly conserved, with Chl *a*/Chl *b* ratios between 1.0 and 1.2 (ref 7). In green algae, the pigment ratio of LHC II is more variable and values between 0.7 and 2.7 have been reported⁹⁻¹¹. We report here that in the unicellular marine chlorophyte,

Dunaliella tertiolecta, the ratio is actually variable. Photoadaptation to high irradiance involves changes in the average composition and behaviour of LHC II; specifically, the Chl *b* content per polypeptide is halved and the efficiency of excitation transfer from carotenoid to Chl *a* declines. The result is a novel mechanism for regulating the effective absorption cross-section of photosystem II.

D. tertiolecta can grow under a wide range of photon flux densities ($10\text{--}2,000\ \mu\text{mol quanta m}^{-2}\text{ s}^{-1}$, 400–700 nm). As in other chlorophytes, cellular chlorophyll content decreases and the Chl *a*/Chl *b* ratio increases as cells adapt to higher growth irradiance levels (Table 1). But even at the relatively low irradiance level of $70\ \mu\text{mol quanta m}^{-2}\text{ s}^{-1}$, the Chl *a*/Chl *b* ratio of *D. tertiolecta* cells is more than twice that recorded for pea seedlings¹² exposed to the same light conditions (5.4 compared with 2.4). The high ratio is not due to an inability to form LHC II, because analysis of thylakoid proteins by SDS-PAGE followed by staining or radioimmune blotting (Fig. 1*a*) shows that LHC II is a major membrane component and is approximately equally abundant per unit chlorophyll in HL (high light) and LL (low light) cells. As Chl *b* is more abundant per unit chlorophyll in LL cells than in HL cells (Table 1), the Chl *b*/LHC II ratio should also increase in LL cells. Direct analysis of purified LHC II from HL and LL cells supports this contention (Table 1). The Chl *a*/Chl *b* ratio of LHC II from HL cells is twice that for LHC II from LL cells and more than four times that for LHC II from higher plants⁷ and from the green alga *Chlamydomonas reinhardtii*⁶. The LHC II of HL cells contains the same number of Chl *a* and lutein molecules per apoprotein molecule as LHC II from LL cells, but only half the number of Chl *b* molecules.

Variations in the average pigment content of LHC II would be expected to give changes in the light harvesting ability of photosystem II¹³. The effective absorption cross-section of photosystem II was examined for HL and LL cells *in vivo* by measurements of the effect of flash intensity on the change in chlorophyll fluorescence yield at 20 °C (arising mainly from photosystem II¹⁴). Figure 2 shows that a higher actinic flash intensity was required for 50% saturation of the change in fluorescence yield in HL cells, corresponding to a 30% decline in photosystem II cross-section. This decrease in cross-section is larger than might be expected for the loss of one Chl *b* molecule from a total of seven chlorophyll molecules in LHC II. Furthermore, it is unlikely that the decrease in photosystem II cross section is due to a reduction in the amount of LHC II per reaction centre because the ratio of LHC II polypeptides to photosystem II reaction centres remains constant over the range $50\text{--}1,900\ \mu\text{mol quanta m}^{-2}\text{ s}^{-1}$ (data not shown)¹⁵. An explanation for the additional loss of effective absorption cross-section was therefore sought in the structure of LHC II.

Purified LHC II preparations from HL and LL cells were compared with respect to the relative quantum yield of fluorescence to infer the efficiency of energy transfer from their accessory pigments (Chl *b* and lutein) to Chl *a*. Relative fluorescence yields were calculated by comparing absorption spectra and corrected fluorescence excitation spectra (Fig. 3). Room temperature, steady state, fluorescence emission spectra of

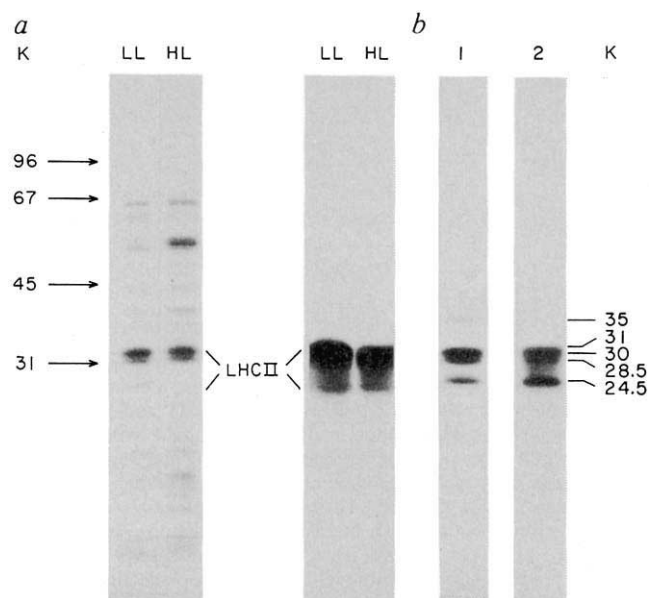


Fig. 1 *a*, Detection of LHC II in thylakoids from cells of *D. tertiolecta* adapted to high light (HL) and low light (LL). Equal chlorophyll concentrations were loaded in both lanes. Left, coomassie blue-stained 10% polyacrylamide gel after SDS-PAGE. Right, replicate gel after radioimmune blotting using anti-pea LHC II antibodies²⁵, which do not cross-react with LHC I²⁶. *b*, 15% SDS-PAGE of purified LHC II (lane 1) and the corresponding radioimmune blot (lane 2) isolated from *D. tertiolecta* grown at $70\ \mu\text{mol quanta m}^{-2}\text{ s}^{-1}$. Thylakoids were prepared as previously described²⁷. All buffers contained $200\ \mu\text{M}$ PMSF. Cells and chloroplasts were ruptured in 0.4 M sucrose and centrifuged at 30,000g for 20 min. The pellet was resuspended and washed three times in 6 mM Tris-glycine, pH 7.8, containing 2 mM EDTA to give $0.5\ \text{mg chlorophyll ml}^{-1}$. Membranes were partially solubilized in 60 mM octyl β -D-glucopyranoside (OG) for 45 min and undissolved membranes were removed by centrifugation at 200,000g for 1 h. The soluble material was loaded on a 0.1–0.5 M linear sucrose gradient containing 2 mM Tris-maleate buffer pH 7.0, 2 mM EDTA and 30 mM OG. The gradients were centrifuged for 16 h at 200,000g and the highly fluorescent green band, which is enriched with LHC II, was collected. The LHC II was further purified by precipitation with 10 mM MgCl_2 and centrifugation for 10 min at 30,000g on a 1 M sucrose cushion. The pellet was resuspended in 2 mM Tris-maleate pH 7.0 with 2 mM EDTA and 30 mM OG.

purified HL and LL LHC II show emission at only one waveband, centred at $680\ \text{nm}$ ¹⁵ which corresponds to Chl *a*. In LHC II from HL cells less than 60% of the light energy absorbed in the 440–520 nm waveband was transferred to Chl *a*, whereas the relative fluorescence yields of LHC II from LL cells were greater than 85% in the same waveband. Similar results were obtained from intact thylakoid membranes, indicating that the loss of energy transfer efficiency at HL is not an artefact of the detergent used to solubilize LHC II.

Table 1 Pigment composition of *D. tertiolecta* and its purified light-harvesting complex under different light conditions

	Cell pigmentation*					LHC II pigmentation*				
	Chl <i>a</i> /cell	Chl <i>b</i> /cell	lutein/cell	Chl <i>a</i> /Chl <i>b</i>	lutein/Chl <i>b</i>	Chl <i>a</i> /LHC	Chl <i>b</i> /LHC	lutein/LHC	Chl <i>a</i> /Chl <i>b</i>	lutein/Chl <i>b</i>
LL	11.4	2.11	5.37	5.4	2.6	4.67	1.92	1.96	2.4	1.0
HL	5.8	0.72	3.33	8.1	4.6	4.95	1.06	1.75	4.7	1.7

* Moles ($\times 10^{-16}$) per cell or mole per mole. LL, low growth irradiance level ($70\ \mu\text{mol quanta m}^{-2}\text{ s}^{-1}$); HL, high growth irradiance level ($1,200\ \mu\text{mol quanta m}^{-2}\text{ s}^{-1}$). Cultures were grown in turbidostats with white fluorescent lights as described²⁴. Pigments were extracted in 80% methanol and analysed by high-performance liquid chromatography on a 3- μm RP-8 column with 95% methanol as the solvent and detected with a spectromonitor at 438 nm and a fluorometer using a mercury light source and equipped with a Corning 2-64 excitation filter and a 680-nm interference filter. Pigments were quantified by areal integration and verified with pure standards isolated from spinach by thin layer chromatography. Protein was determined by amino acid analysis. LHC II was purified as described in Fig. 1.

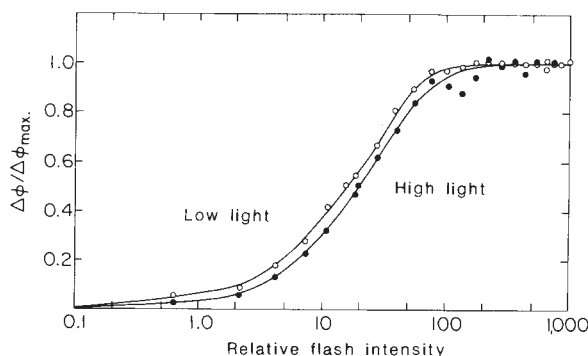


Fig. 2 The flash saturation curves of the change in fluorescence yields of intact cells of *D. tertiolecta* grown at 70 and 1,200 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$. The change in fluorescence yield was measured by the 'pump and probe' technique where flash pairs were triggered at 0.5 Hz with a 75- μs delay between the actinic 'pump' and measuring 'probe' flashes¹⁷. The probe flash intensity was 0.3% of the maximum actinic flash intensity. The change in fluorescence yield ($\Delta\phi$) was calculated from the equation $\Delta\phi = F_s - F_p/F_p$, where F_p is the fluorescence yield of the probe flash alone and F_s is yield of the probe flash following an actinic flash. The flash saturation curves were fitted to a cumulative one-hit Poisson distribution, where $\Delta\phi/\Delta\phi_{\text{max}} = 1 - e^{-\sigma E}$, E is relative flash intensity and σ is the relative effective absorption cross-section of photosystem II²⁸.

We conclude that *D. tertiolecta* can form LHC II complexes with Chl *a*/Chl *b* ratios that are much higher than the ratios seen in higher plants. Moreover, we report for the first time that a green alga can increase this ratio in response to increasing growth irradiance. Accompanying the change in ratio is a decline in the efficiency with which the remaining accessory pigments transfer excitation energy to Chl *a*. This appears to contribute to a loss in the effective absorption cross-section of photosystem II. A model for the structure of higher plant LHC II envisions a central core of three interacting Chl *b* molecules per complex⁶. Our data suggest that loss of one or two of these molecules may alter the energy transfer properties of the remaining accessory pigments. Assuming that reduction in energy transfer efficiency from Chl *b* would result in steady-state room-temperature fluorescence emission at 650 nm (which was not observed) we infer that the remaining Chl *b* transfers excitation energy with very high efficiency to Chl *a* in the intact HL complex. There is, however, an overall reduction in energy transfer efficiency in the complex, which suggests that lutein is inefficiently coupled to the remaining pigments (Fig. 3). The 30% reduction in effective absorption cross-section would clearly be advantageous to cells exposed to excessively high irradiance levels, given the sensitivity of photosystem II to photoinhibition¹⁹.

Three other specific mechanisms for protecting photosystem II from the deleterious effects of high irradiance have been advanced. They are: (1) reduction in photosystem II cross-section following phosphorylation of LHC II⁵; (2) dissipative cyclic electron flow around photosystem II¹⁷; again possibly modulated by a protein phosphorylation¹⁸; and (3) resynthesis of D1, a photosystem II core protein of relative molecular mass 32,000 (M_r 32K), which may suffer damage as a result of electron transport¹⁹. Photoinhibition at high irradiance involves loss of protein kinase activity and D1 content^{20,21}; both recover only after removal of photoinhibitory conditions. Thus, the above three mechanisms might delay, but not prevent, the onset of photoinhibition. The mechanism we describe here is a slower adaptive mechanism which could reduce energy input into PSII by as much as 30%. Both the reduction in Chl *b* content and the screening effect of poorly-connected auxiliary pigments should diminish the probability of photoinhibition.

How a variable Chl *a*/Chl *b* ratio for LHC II is achieved is not yet clear. LHC II from *D. tertiolecta* contains at least five

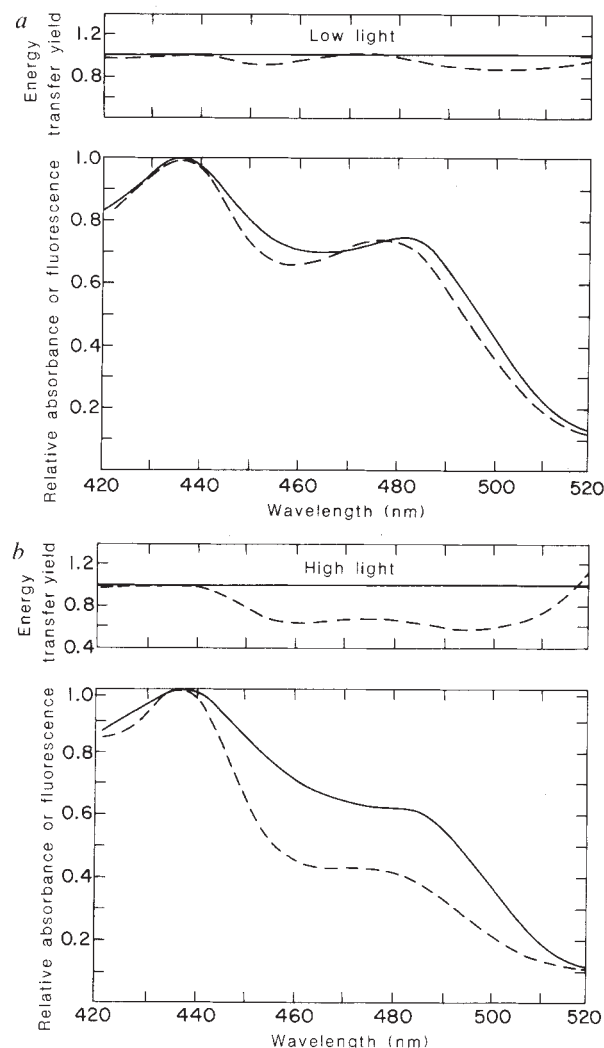


Fig. 3 Fluorescence yields, normalized room-temperature absorption and corrected fluorescence excitation spectra for purified LHC II solubilized in 30 mM octyl- β -D-glucopyranoside. *a*, LHC II from low-light-grown cells; and *b*, LHC II from high-light-grown cells. Absorption spectra (solid lines) were measured with an SLM-Aminco DW2c spectrophotometer and fluorescence excitation spectra (dotted lines) were measured with a SLM 4800S spectrofluorometer. Emission was measured at 680 nm. Relative energy transfer yields (top panels) were calculated from the ratios of the fluorescence excitation and the corresponding absorption spectra, normalized at 435 nm. In the two preparations, the absolute fluorescence yields at 435 nm were equal and have been set at unity for the calculation of transfer efficiency.

distinct apoproteins with M_r s of 24.5, 28.5, 30.0, 31.0, and 35K (Fig. 1b). As each of these could bind a different number of Chl *a* and Chl *b* molecules, changes in the relative amount of the apoproteins could generate a range of Chl *a*/Chl *b* ratios without changing the LHC II/photosystem II ratio. A second possibility is that the pigment composition of LHC II is controlled at the level of pigment availability. Although studies on intermittently illuminated plants and on a Chl *b*-less mutant of barley have led to the concept that LHC II apoproteins are degraded in the absence of pigments⁴, two exceptions to this rule are known: the LHC II apoproteins of *C. reinhardtii* accumulate at approximately normal levels in a Chl *b*-less mutant²²; and one particular LHC II apoprotein of the barley mutant accumulates normally²³. We speculate that the LHC II apoproteins which lack their full complement of Chl *b* may be stable in HL cells of *D. tertiolecta*, but may be responsible for the inefficient energy transfer detected here. Preparation of

monoclonal antibodies specific for individual LHC II apoproteins should provide a means for assaying each apoprotein in cells exposed to different growth irradiances. But to relate any changes in the apoprotein levels to the variation in Chl *a*/Chl *b* ratios, it will also be necessary to isolate individual LHC II complexes and determine their pigment composition.

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1. Drews, G. *Trends Biochem. Sci.* **11**, 255-257 (1981).
2. Falkowski, P. G. in *Primary Productivity in the Sea* (ed. Falkowski, P. G.) 99-119 (Plenum, New York, 1980).
3. Boardman, N. K. A. *Rev. Pl. Physiol.* **28**, 355-377 (1977).
4. Bennett, J. *Biochem. J.* **212**, 1-13 (1983).
5. Staehelin, L. A. & Arntzen, C. J. *J. cell Biol.* **97**, 1327-1337 (1983).
6. Kan, K.-S. & Thornber, J. P. *Pl. Physiol.* **57**, 47-52 (1976).
7. Anderson, J. M. A. *Rev. Pl. Physiol.* **37**, 93-136 (1986).
8. Leong, T.-Y. & Anderson, J. M. *Biochem. biophys. Acta* **850**, 57-63 (1986).
9. Apel, K. *Brookhaven Symp. Biol.* **28**, 149-161 (1977).
10. Cunningham, F. & Shiff, J. A. *Pl. Physiol.* **80**, 223-230 (1986).
11. Anderson, J. M., Waldron, J. C. & Thorne, S. W. *Pl. Sci. Lett.* **17**, 149-151 (1980).
12. Leong, T.-Y. & Anderson, J. M. *Photosynth. Res.* **5**, 105-115 (1984).
13. Herron, H. A. & Mauzerall, D. *Pl. Physiol.* **50**, 141-148 (1972).
14. Bradbury, M. & Baker, N. R. *Biochim. biophys. Acta* **635**, 562-551 (1981).
15. Sukenik, A., Bennett, J. & Falkowski, P. G. *Biochim. biophys. Acta* (submitted).
16. van Metter, P. L. *Biochim. biophys. Acta* **462**, 642-658 (1977).
17. Falkowski, P. G., Fujita, Y., Ley, A. & Mauzerall, D. *Pl. Physiol.* **81**, 310-312 (1986).
18. Lee, P. & Horton, P. *FEBS Lett.* **162**, 81-84 (1983).
19. Kyle, D. J. *Photochem. Photobiol.* **41**, 107-116 (1985).
20. Kyle, D. J., Ohad, I. & Arntzen, C. J. *Proc. natn. Acad. Sci. U.S.A.* **81**, 4070-4074 (1984).
21. Schuster, G., Dewitt, M., Staehelin, L. A. & Ohad, I. *J. cell Biol.* **103**, 71-80 (1986).
22. Michel, H. P., Tellenbach, M. & Boschetti, A. *Biochim. biophys. Acta* **725**, 417-424 (1983).
23. Darr, S. C., Somerville, S. C. & Arntzen, C. J. *J. Cell Biol.* **103**, 733-740 (1986).
24. Falkowski, P. G. *Photosynthetica* **18**, 62-62 (1984).
25. Bennett, J., Jenkins, G. I. & Hartley, M. R. *J. cell. Biochem.* **25**, 1-13 (1984).
26. Williams, R. S. & Ellis, R. J. *FEBS Lett.* **203**, 295-300 (1986).
27. Williams, R. S., Shaw, E. K., Sieburth, L. E. & Bennett, J. *Meth. Enzym.* **118**, 338-352 (1986).
28. Falkowski, P. G., Wyman, K. D., Ley, A. C. & Mauzerall, D. C. *Biochim. biophys. Acta* **849**, 183-192 (1986).

Electrogenic glutamate uptake is a major current carrier in the membrane of axolotl retinal glial cells

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Glutamate is taken up avidly by glial cells in the central nervous system¹. Glutamate uptake may terminate the transmitter action of glutamate released from neurons¹, and keep extracellular glutamate at concentrations below those which are neurotoxic. We report here that glutamate evokes a large inward current in retinal glial cells which have their membrane potential and intracellular ion concentrations controlled by the whole-cell patch-clamp technique². This current seems to be due to an electrogenic glutamate uptake carrier, which transports at least two sodium ions with every glutamate anion carried into the cell. Glutamate uptake is strongly voltage-dependent, decreasing at depolarized potentials: when fully activated, it contributes almost half of the conductance in the part of the glial cell membrane facing the retinal neurons. The spatial localization, glutamate affinity and magnitude of the uptake are appropriate for terminating the synaptic action of glutamate released from photoreceptors and bipolar cells. These data challenge present explanations of how the b-wave of the electroretinogram is generated, and suggest a mechanism for non-vesicular voltage-dependent release of glutamate from neurons.

Application of L-glutamate to glial (Müller) cells isolated³ from axolotl retina evoked an inward current (Fig. 1a). The current decreased towards zero with polarization to positive

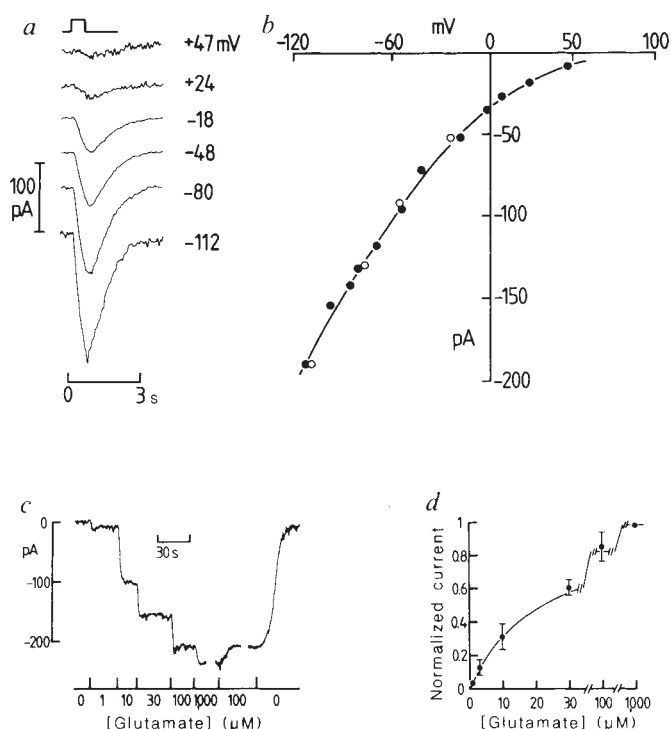


Fig. 1 Dependence of the glutamate uptake current on voltage (a, b) and glutamate concentration (c, d). a, Inward currents evoked by glutamate iontophoresis in a Müller cell in 6 mM barium-Ringer's, voltage-clamped to the potential shown next to each record. Top trace, iontophoresis timing. b, Peak currents from the cell in a (●) and from another cell (○) in normal Ringer's (see below) as a function of voltage. Even near +50 mV (the Nernst potential for sodium ions) the current is still inward. At potentials between +60 mV and +100 mV no glutamate-evoked current was detectable (data not shown). Asymptotic approach of the current towards zero was also seen with lowered external sodium concentration (Fig. 2b). Although the concentration of glutamate around the cell is non-uniform in this iontophoretic experiment, the shape of the current-voltage relation is independent of the concentration of glutamate (see text). c, Current evoked in a cell voltage-clamped to -80 mV by different concentrations of superfused glutamate (shown at the bottom). A detectable current was evoked by 1 μM glutamate. In other experiments 2 mM glutamate was shown to evoke the same current as did 1 mM. d, Average dose-response curve from experiments like that in c on 10 cells at -40 mV (data from at least three cells for each point). At low doses, the current is proportional to glutamate concentration. Curve through the points is a Michaelis-Menten relation (current = [glutamate]/([glutamate] + K_m)) with K_m = 22 μM.

Methods. Glial cells (Müller cells) were isolated from the axolotl retina by papain dissociation³, and recorded from using the whole-cell variant of the patch-clamp technique². Pipettes contained (mM): KCl 80; potassium acetate 15; NaCl 5; HEPES 5; MgCl₂ 7; Na₂ATP 5; CaCl₂ 1; K₂EGTA 5; pH adjusted to 7.0 with KOH. The glutamate-evoked current was still present if ATP was omitted from the pipette. Normal Ringer's contained (mM): NaCl 105; KCl 2.5; CaCl₂ 3; MgCl₂ 0.5; glucose 15; HEPES 5; pH adjusted to 7.25 with NaOH. Barium-Ringer's had 6 mM BaCl₂ added to this to block the resting K⁺ conductance of the cell¹⁵. Experiments were usually carried out in barium-Ringer's, because of the greater voltage range which could then be studied. Extracellular barium had no effect on the magnitude of the glutamate-evoked current. External sodium concentrations lower than that in normal Ringer's (107 mM) were obtained by replacing NaCl with choline chloride. Addition of 43 mM NaCl was used to obtain [Na⁺]_o of 150 mM (as in Fig. 2). Glutamate or other drugs were added to the external solution. Glutamate iontophoresis was from pipettes filled with 1 M sodium glutamate (resistance 30 MΩ in Ringer's). Typically, a holding current of +30 nA was switched to -40 nA for ejection.

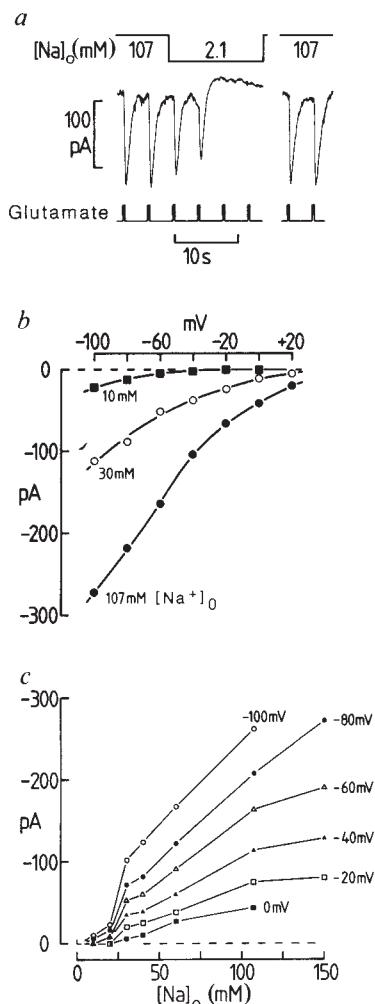


Fig. 2 Sodium-dependence of the glutamate-evoked current. *a*, Glutamate was repeatedly applied iontophoretically (bottom trace) to a cell clamped at -85 mV. The inward current thus evoked was rapidly and reversibly abolished when the barium-Ringer's superfusing the cell was replaced with barium-Ringer's containing only 2.1 mM $[\text{Na}^+]_o$ (solution change takes ~ 6 s). *b*, Voltage-dependence of the current evoked by 30 μM superfused glutamate in solutions containing 107 mM $[\text{Na}^+]_o$ (normal barium-Ringer's), or 30 or 10 mM $[\text{Na}^+]_o$. Data all from one cell. *c*, Dependence on $[\text{Na}^+]_o$ of the current evoked by 30 μM superfused glutamate at different membrane potentials (given by each curve). Mean data obtained from 12 cells. Glutamate-evoked inward current is plotted upwards.

potentials (Fig. 1*b*), but even at $+100$ mV (50 mV more positive than the Nernst potential for sodium ions) no reversal of the current was seen. Glutamate produced no detectable increase of noise in the membrane current (0.5 – 500 Hz variance/mean current < 0.005 pA at -40 mV). These results suggest that the inward current reflects activation of an electrogenic carrier by externally applied glutamate, rather than opening of ion channels. Pharmacological evidence presented here indicates that this is the glutamate uptake carrier.

Figure 1*c* and *d* shows the dependence of glutamate-evoked current on glutamate concentration. The dose-response curve is linear at low doses and can be roughly fitted by a Michaelis-Menten curve between 1 μM and 1 mM, suggesting that one glutamate anion binds to the carrier. The dependence of the current on the concentration of glutamate was independent of voltage: fitting a Michaelis-Menten relation to data like those in Fig. 1*d* gave an apparent K_m of ~ 20 μM between -100 and -20 mV. This implies that the current-voltage (I – V) relation is

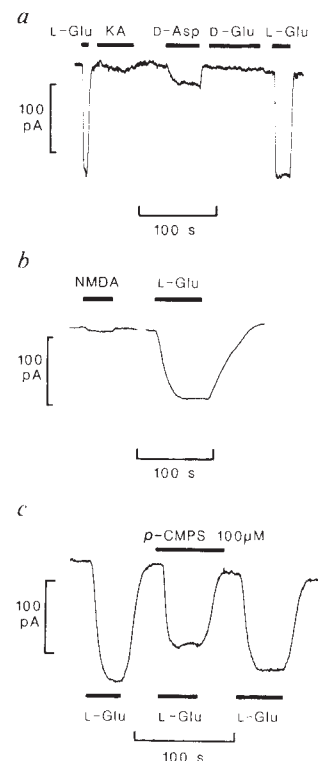


Fig. 3 Pharmacology of the glutamate uptake carrier. *a*, *b*, Comparison of the effects of glutamate analogues: all drug concentrations were 30 μM ; solid bars, superfusion periods. *a*, Effects of successively L-glutamate (L-Glu), kainic acid (KA), D-aspartate (D-Asp), D-glutamate (D-Glu) and L-glutamate again on a cell clamped at -40 mV. *b*, Effects of *N*-methyl-D-aspartate (NMDA) and L-glutamate on a cell clamped at -40 mV. No current was evoked by quisqualate (30 μM , data not shown). *c*, Effect of *p*-chloromercuriphenylsulphonate (*p*-CMPS) (top bar, application period) on the current evoked by 30 μM L-glutamate in a cell clamped at -42 mV. The blocker threo-3-hydroxy-DL-aspartate (30 μM) also reduced the current evoked by 30 μM glutamate (by about 80% at -40 mV).

simply scaled in amplitude, with no change in shape, by changes of glutamate concentration.

Decreasing the external sodium concentration, $[\text{Na}^+]_o$, reduced the glutamate-induced inward current and in 2.1 mM $[\text{Na}^+]_o$ the response was abolished (Fig. 2*a*). Figure 2*b* shows the voltage-dependence of the current induced by 30 μM glutamate for three different values of $[\text{Na}^+]_o$. Decreases of $[\text{Na}^+]_o$ change the shape of the I – V relation: the current is reduced by a larger fraction at depolarized than at hyperpolarized potentials, indicating a lower apparent affinity for sodium at depolarized potentials. Figure 2*c* shows the current evoked by 30 μM glutamate as a function of $[\text{Na}^+]_o$ at different voltages (averaged data from 12 cells). For membrane potentials below -40 mV, the current is approximately proportional to $([\text{Na}^+]_o)^n$ below 40 mM $[\text{Na}^+]_o$, with n between 1.9 (at -100 mV) and 2.5 (at -40 mV), suggesting that at least two sodium ions must bind to the carrier activated by glutamate. Co-transport of two or more Na^+ with each glutamate anion would account for the inward current evoked by glutamate. Co-transport of sodium ions is thought to provide the energy needed for accumulation of glutamate against a concentration gradient⁴.

Analogues of L-glutamate also evoked an inward current in Müller cells (Fig. 3*a* and *b*). At a concentration of 30 μM , the currents evoked at -40 mV by L-glutamate, L-aspartate, D-aspartate, NMDA (*N*-methyl-D-aspartate), kainate, quisqualate and D-glutamate had relative magnitudes $1:0.43:0.25:0.06:0.005:0:0$ (mean data from three cells). Even at 1 mM, these analogues evoked currents smaller than that

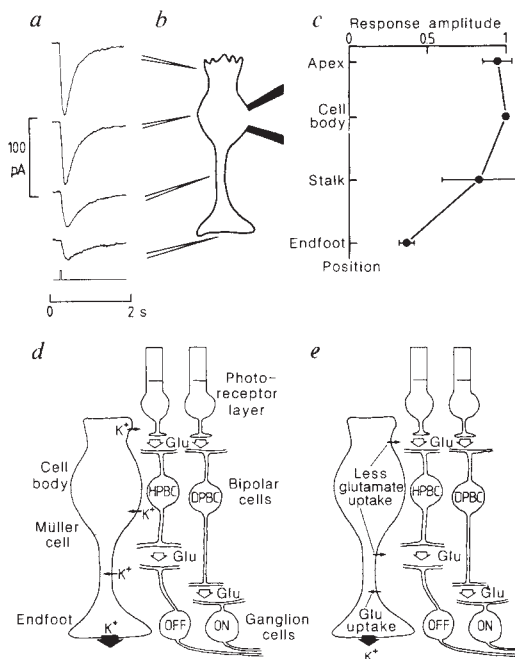


Fig. 4 Spatial distribution of glutamate uptake (*a, c*) and its contribution to the electroretinogram (*d, e*). *a*, Responses to glutamate iontophoresed 10 μ m away from a cell at the positions shown in *b*. Holding potential -42 mV. The response at the endfoot (25 pA amplitude, time-to-peak 180 ms) was not due to glutamate diffusing to the cell body: positioning the iontophoretic pipette at the same distance sideways from the cell body, as the endfoot is lengthways, evoked only a 4-pA response with a time-to-peak of 800 ms (reflecting the long diffusion distance). *c*, Mean amplitudes (normalized to the value at the soma) of the responses to glutamate at different cell positions. Data from five cells. *d*, Schematic diagram of the retina showing the conventional explanation for how the b-wave of the electroretinogram is generated. Light hyperpolarizes photoreceptors, causing K⁺ to enter them and thus decreasing [K⁺]_o in the outer retina. Light-induced depolarization of neurons in the inner retina causes K⁺ efflux from those cells, and thus raises [K⁺]_o in the inner retina. As a result, K⁺ flows out of the Müller cell in the outer retina and flows into it in the inner retina. Any net current thus produced flows across the endfoot membrane of the cell, which has over 90% of the potassium conductance of the cell^{3,7}. The resulting current flow along the Müller cell generates a trans-retinal voltage which can be recorded outside the eye¹². *e*, Contribution of changes in glutamate uptake to the b-wave. Light hyperpolarizes photoreceptors and hyperpolarizing bipolar cells (HPBC) and thus decreases glutamate release from their synaptic terminals; light depolarizes depolarizing bipolar cells (DPBC), increasing glutamate release from their synaptic terminals⁸⁻¹⁰. Consequently, glutamate uptake into Müller cells decreases in the outer retina and increases in the inner retina (DPBCs terminate deeper in the retina than HPBCs). Any net current change thus generated flows across the high (potassium) conductance membrane in the endfoot.

produced by 30 μ M L-glutamate. This pharmacological profile suggests that the current is caused by the activation of an electrogenic glutamate transporter rather than a glutamate-activated channel⁴⁻⁶. A glutamate uptake blocker, *p*-chloromercuriphenylsulphonate (100 μ M), reduced the glutamate-evoked current by about 30% (Fig. 3c), which is similar to other estimates of the effectiveness of this blocker⁶.

The spatial distribution of the glutamate-activated carrier was studied by iontophoresing glutamate onto different regions of isolated Müller cells (Fig. 4a-c). The current evoked was similar at the apical membranes, cell body and stalk of the cell, that is, the parts of the cell membrane facing the neurons of the retina, but smaller at the terminal endfoot membrane, facing the vitreous humour. This distribution contrasts dramatically with that of the potassium channels in the cell membrane, over 90%

of which are in the endfoot^{3,7}. The high apparent affinity of the carrier for glutamate and its presence in membranes facing the retinal neurons are appropriate for removing glutamate from the extracellular space to terminate synaptic transmission: glutamate is a candidate for the neurotransmitter released from retinal photoreceptors and bipolar cells⁸⁻¹⁰. Quantitatively, the velocity of glutamate uptake is sufficient to carry out this function. At a resting potential of -90 mV, 30 μ M glutamate evokes an uptake current of approximately 236 pA (Fig. 2c): this current (assumed to reflect the transport of two Na⁺ ions per glutamate anion) is sufficient to clear the extracellular space around each Müller cell of 30 μ M glutamate in 80 ms, which is comparable to the timescale on which retinal synapses operate¹¹. (Here the extracellular space was taken as 7% (ref. 12) of the volume of neural retina per Müller cell, that is 7% of 9×10^{-14} m³ for 100 μ m long cells spaced 30 μ m apart).

The glutamate uptake carrier can generate a significant current across the glial cell membrane. When fully activated, the slope conductance of its *I*-*V* relation near the normal resting potential of -90 mV is ~ 5 nS (from Figs 2c and 1d), similar to the K⁺ conductance³ (7 nS) in the part of the cell facing the neural retina where the uptake carrier is mainly located.

This has implications for our understanding of the electroretinogram, a potential change which can be recorded outside the eye and is used clinically. When light is applied to the retina, the resulting changes in glutamate release from retinal neurons⁸⁻¹⁰ will alter glutamate uptake into Müller cells (Fig. 4e). The spatial distribution of the resulting changes expected in the Müller cell membrane current is similar to that generated by light-induced changes in [K⁺]_o around the Müller cell (Fig. 4d). Radial current flow along Müller cells produced by these [K⁺]_o changes is believed to generate the b-wave of the electroretinogram¹². Our data suggest that changes in glutamate uptake contribute to shaping the b-wave, because the changes in uptake current could easily be larger than the currents generated by [K⁺]_o changes. A change in glutamate concentration from 1 to 100 μ M around a Müller cell generates a current change of 320 pA at a resting potential of -90 mV (Figs 2c and 1d). For comparison, a twofold rise in [K⁺]_o, which is much greater than the largest rise seen experimentally¹², around the part of the cell facing the retinal neurons would generate a current of 125 pA.

Depolarization-induced release of glutamate from retinal neurons is known to occur in the absence of extracellular calcium⁸. This release, which may be non-vesicular, could be accounted for by the strong voltage-dependence transport which we have observed, if the same carrier exists in neurons and if the glutamate concentration in neurons is high enough to allow a net glutamate efflux on depolarization.

We looked for glutamate uptake in axolotl retinal glia because of the importance of glutamate as a retinal neurotransmitter in lower vertebrates. Since glutamate depolarizes astrocytes from rat brain^{13,14} the electrogenic mechanism characterized here may be of general importance.

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- Hertz, L. *Prog. Neurobiol.* **13**, 277-323 (1979).
- Hamill, O. P., Marty, A., Neher, E., Sakmann, B. & Sigworth, F. J. *Pflügers Arch. ges. Physiol.* **391**, 85-100 (1981).
- Newman, E. A. *Nature* **309**, 155-157 (1984).
- Stallcup, W. B., Bulloch, K. & Baetge, E. E. *J. Neurochem.* **32**, 57-65 (1979).
- Watkins, J. C. & Evans, R. H. A. *Rev. Pharmac. Tox.* **21**, 165-204 (1981).
- Balcar, V. J. & Johnston, G. A. R. *J. Neurochem.* **19**, 2657-2666 (1972).
- Brew, H., Gray, P. T. A., Mobbs, P. & Attwell, D. *Nature* **324**, 466-468 (1986).
- Miller, A. M. & Schwartz, E. A. *J. Physiol., Lond.* **334**, 325-350 (1983).
- Cervetto, L. & MacNichol, E. F. *Jr Science* **178**, 767-768 (1972).
- Slaughter, M. M. & Miller, R. F. *J. Neurosci.* **3**, 1701-1711 (1983).
- Schnapf, J. L. & Copenhagen, D. R. *Nature* **296**, 862-864 (1982).
- Newman, E. A. & Odette, L. L. *J. Neurophysiol.* **51**, 164-183 (1984).
- Bowman, C. L. & Kimelberg, H. K. *Nature* **311**, 656-659 (1984).
- Kettenmann, H. & Schachner, M. *J. Neurosci.* **5**, 3295-3301 (1985).
- Newman, E. A. *Nature* **317**, 809-811 (1985).

An AIDS-related cytotoxic autoantibody reacts with a specific antigen on stimulated CD4⁺ T cells

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Patients with the acquired immune deficiency syndrome (AIDS) and AIDS-related conditions are known to have abnormalities of T cell subpopulations, including a decreased helper/inducer (bearing the CD4 antigen) to suppressor/cytotoxic (bearing the CD8 antigen) T cell ratio and decreased absolute numbers of T cells with the CD4⁺ phenotype^{1,2}. Infection of T cells with a retrovirus, termed human immunodeficiency virus (HIV), is thought to be important in these abnormalities³⁻⁵. HIV infection alone does not adequately explain the CD4⁺ T-cell abnormalities seen in AIDS, however, and the nature of T-cell destruction in this disease remains poorly characterized⁶. Here we describe an AIDS-related serum autoantibody that reacts with an antigen of relative molecular mass 18,000 (*M_r* 18K) restricted to lectin-stimulated or HIV-infected CD4⁺ T cells. The antibody also suppresses proliferation of CD4⁺ T cells *in vitro* and induces cytotoxicity of these cells in the presence of complement. Its role in the development of AIDS merits attention.

A number of autoimmune phenomena have been described in patients with AIDS and AIDS-related conditions⁷⁻¹⁵. Serum autoantibody against a 25K platelet protein has been found in 95% of homosexual men with immune thrombocytopenic purpura (ITP), as well as in 80% of homosexual patients with AIDS⁷. The lupus anticoagulant (anti-phospholipid antibody), autoantibodies against red blood cells, and anti-nuclear antibodies have been detected in homosexual and haemophilic patients with AIDS and AIDS-related conditions⁸⁻¹². In addition, anti-lymphocyte antibodies have been reported in these patients¹³⁻¹⁹. Kiprov *et al.*¹³ found antibodies against T cells in 70% of patients with AIDS. These antibodies reacted with 60% of helper/inducer T cells but were unreactive with suppressor/cytotoxic T cells. Dorsett *et al.*¹⁸ also found selective binding of serum antibody from AIDS patients to OKT-4-positive (helper/inducer) T cells. A possible target T-cell antigen has been suggested by Belisto *et al.* and others²⁰⁻²², who postulated that an aberrant autoimmune response in AIDS patients may be directed against the highly polymorphic HLA-DR region found on both Langerhans epithelial cells and lymphocytes. Evidence in favour of this hypothesis is lacking at present²³.

To characterize antibody binding to T cells in AIDS, we obtained serum samples from 80 subjects (Table 1). The control group of 41 patients included 10 heterosexual subjects (7 women and 3 men) with ITP, 17 asymptomatic homosexual men and 14 healthy haemophiliacs. None of the heterosexual controls had serum antibody against HIV, as determined by an indirect immunofluorescence assay (IFA) and immunoblotting^{3,24}. Seventeen of the homosexual and haemophilic controls were negative for serum anti-HIV antibody, whereas 14 were HIV antibody-positive. The patient group consisted of 27 homosexual men and 12 haemophiliacs (11 men and 1 woman) with lymphadenopathy, ITP or AIDS. Each of these patients had serum antibody against HIV. All AIDS patients met the criteria for the disease established by the Centers for Disease Control²⁵.

The mean helper/suppressor T-cell ratio of the 17 homosexual controls was 1.8 (range 1.2-3.5), whereas the mean T-cell ratio of the patients with AIDS and AIDS-related conditions was 0.3 (range 0.1-0.9), as determined using monoclonal antibodies and

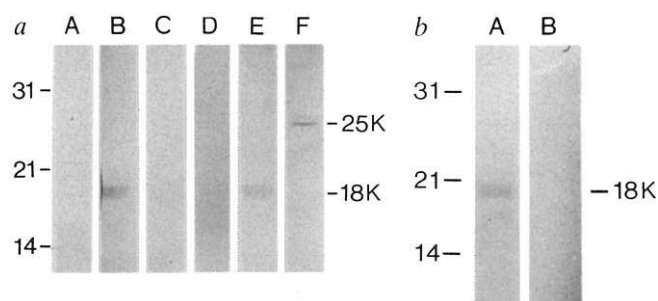


Fig. 1 *a*, Detection of anti-T-cell antibody by immunoblotting. Serum from a homosexual man with ITP was incubated with: A, unstimulated peripheral blood T cells; B, PHA-stimulated uninfected T cells; C, B cells; D, monocytes; E, T cells infected with HIV after isolation from peripheral blood; F, platelets. Serum antibody bound to an 18K protein on stimulated or infected T cells (B and E) and to a previously described 25K platelet antigen (F)⁷. *b*, Specificity of antibody binding to CD4⁺ cells. Serum antibody from a homosexual AIDS patient bound to the 18K antigen on stimulated CD4⁺ cells (A), but not on stimulated CD8⁺ cells (B). When T-cell proteins were subjected to disulphide bond reduction using dithiothreitol before SDS-PAGE and immunoblotting, antibody binding to the 18K protein on CD4⁺ cells was no longer detected (data not shown).

Methods. *a*, Human T cells were obtained from control blood by Ficoll-Hypaque separation followed by sheep red-cell rosetting³⁵. The cells were grown in the presence or absence of phytohaemagglutinin (PHA), 4 μ g ml⁻¹ for 5 days and then collected and solubilized in 2% sodium dodecyl sulphate (SDS) sample buffer at 5 $\times$ 10⁷ cells ml⁻¹ (5 mg ml⁻¹). B cells were collected after T-cell separation and solubilized at the same concentration in 2% SDS. Monocytes were separated by adherence to plastic plates³⁵, and granulocytes were prepared by sucrose gradient separation³⁵. These cells were solubilized in 2% SDS at 5 mg ml⁻¹. Platelets were prepared as previously described⁷ and solubilized at 5 $\times$ 10⁹ cells ml⁻¹ (5 mg ml⁻¹). Immunoblotting was performed as previously described^{7,36}. Briefly, 50 μ l of the target cell preparations were subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) using 12% gels under non-reducing conditions. Pre-stained molecular weight standards (BRL) were electrophoresed with the samples. After transfer to nitrocellulose and blocking with 5% gelatin in Tris-buffered saline (TBS), the nitrocellulose was incubated with serum, F(ab)₂ fragments (3 mg ml⁻¹) or T-cell eluates (1 mg ml⁻¹) diluted 1:50 in TBS containing 1% gelatin. Antibody binding was detected using a biotin-avidin-peroxidase system, as previously described⁷. Biotinylated F(ab)₂ goat-anti-rabbit IgG was used to detect rabbit antibody binding; biotinylated F(ab)₂ goat anti-human κ/λ light chain was used to detect patient F(ab)₂ binding on the nitrocellulose. *b*, Freshly isolated peripheral-blood T cells were separated by panning³⁷ into Leu-3-positive (CD4⁺) and Leu-2-positive (CD8⁺) populations, as defined by monoclonal antibodies (Beckton-Dickinson). The purity of each population was >90%, as determined by flow cytometry using these antibodies. The cells were grown in PHA for 5 days and solubilized in SDS at 5 $\times$ 10⁷ cells ml⁻¹.

flow cytometry¹⁹. All the individuals with ITP had elevated platelet-associated immunoglobulin, as demonstrated by a flow cytometric technique⁷. The 6 homosexual men with ITP and 10 of 13 homosexual AIDS patients who were studied had serum antibody against a 25K platelet protein, as previously described⁷. This antibody was not detected in sera from any of the heterosexual or haemophilic patients with ITP (data not shown).

Using a sensitive immunoblot technique, we found that patients with AIDS and AIDS-related conditions produce a serum antibody that binds to an antigen on normal lectin-stimulated T cells and on HIV-infected T cells (Fig. 1*a*). The antibody was not detected in sera from 40 of 41 controls, but it was found in sera from 37 of 39 patients with AIDS and AIDS-related conditions (Table 1). The target T-cell protein was not found on unstimulated or uninfected T cells or other blood cells (Fig. 1*a*) including granulocytes (not shown). The antigen

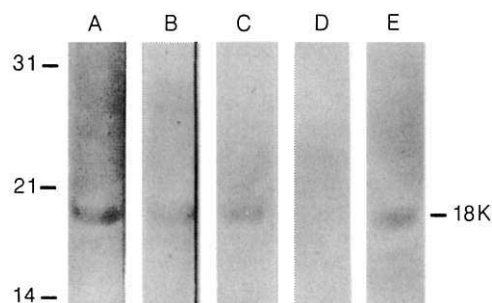


Fig. 2 Binding of serum antibody, $F(ab)_2$ fragments and T-cell eluates to the human helper/inducer T-cell line, HUT-78 (ref. 3). Binding of serum antibody from a homosexual ITP patient to: A, PHA-stimulated HUT-78 cells; B, HIV-infected HUT-78 cells. Unstimulated, uninfected HUT-78 cells did not express the 18K antigen (not shown). C, Binding of $F(ab)_2$ fragments (3 mg ml^{-1}) from a homosexual ITP patient to PHA-stimulated, HIV-infected HUT-78 cells. Binding of T-cell antibody eluate (1 mg ml^{-1}) from a haemophilic AIDS patient to: D, unstimulated HUT-78 cells and E, PHA-stimulated HUT-78 cells. This antibody eluate also detected an 18K antigen on $CD4^+$ PHA-stimulated control T cells (data not shown).

Methods. Uninfected HUT-78 cells were grown in the presence or absence of PHA ($4 \mu\text{g ml}^{-1}$) for 5 days and solubilized at the same concentration as fresh T cells (Fig. 1). Non-PHA-stimulated HUT-78 cells infected with HIV_{SF2} (formerly AIDS-associated retrovirus-2) were grown under the same conditions. Viral infection was demonstrated using a reverse transcriptase assay³. Purified IgG and $F(ab)_2$ fragments were prepared from the serum of homosexual ITP and AIDS patients as previously described⁷. The $F(ab)_2$ fragments at a concentration of 3 mg ml^{-1} were shown to be free of residual IgG by SDS-PAGE. Serum incubation with lectin-stimulated HUT-78 cells and subsequent antibody elution was performed by the acid elution technique described by Falus *et al.*³⁸. PHA-stimulated HUT-78 cells (1×10^7) were incubated with 1 ml of control or AIDS serum for 1 h at room temperature. The cells were washed in phosphate-buffered saline (PBS), and antibody was eluted using 0.1 M glycine, pH 2.5. The eluate was neutralized with 1 M Tris and dialysed against PBS. Analysis of the eluates by SDS-PAGE revealed two bands corresponding to IgG and albumin (data not shown). The IgG concentration in the eluates was determined by a standard rate nephelometric technique³⁹. Immunoblotting was performed as described in Fig. 1.

is a protein of M_r 18K that appears to be restricted to stimulated T cells of the $CD4^+$ phenotype (Fig. 1b). It was also detected on lectin-stimulated or virus-infected cells from the human helper/inducer T-cell line HUT-78 (Fig. 2). The antigen is expressed on the T-cell surface, as antibody eluted from stimulated HUT-78 cells bound to the 18K protein on immunoblots (Fig. 2). The antibody was shown to bind via the $F(ab)_2$ portion to autologous HIV-infected T cells (Figs 2 and 3), indicating autoantibody rather than immune complex binding to the target antigen. The target T-cell protein appears to be distinct from the 25K platelet protein recognized by serum antibody from homosexual men with ITP (Fig. 1a). It also appears to be an integral T-cell protein that is distinct from HLA antigens or HIV antigens defined by available immunoblot reagents (Fig. 3).

We also demonstrated that IgG fractions of AIDS serum containing the anti-T-cell autoantibody selectively inhibited $CD4^+$ T-cell proliferation *in vitro* (Fig. 4a). Virus-free purified IgG from AIDS patients inhibited proliferation of lectin-stimulated, uninfected $CD4^+$ cells by 58–62%. No inhibitory effect of the IgG on proliferation of lectin-stimulated $CD8^+$ cells was seen (Fig. 4a). Furthermore, HUT-78 cell antibody eluates from AIDS patients were cytotoxic for 60–70% of lectin-stimulated $CD4^+$ cells in the presence of complement. $CD8^+$ cells were unaffected by the eluates (Fig. 4b). These findings suggest that anti-T-cell autoantibody may be important in the development of selective T-cell abnormalities in AIDS. Furthermore, the

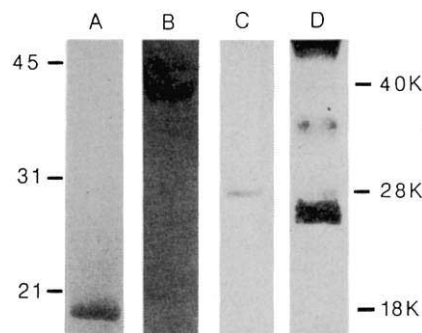


Fig. 3 Characterization of the target T-cell protein in relation to other cellular antigens. A, serum antibody from a haemophilic AIDS patient detects the 18K protein on autologous HIV-infected T cells. B, rabbit antiserum detects the 40K HLA class I (ABC) framework antigen on PHA-stimulated, HIV-infected T cells; C, rabbit antiserum detects the 28K HLA class II (DR) framework antigen on the same cells. The 18K protein was not recognized by antibody specific for β_2 -microglobulin (M_r 12K) (not shown). D, rabbit anti-HIV antiserum binding to sucrose-purified HIV proteins (core, envelope, polymerase) immobilized on nitrocellulose.

Methods. Serum and autologous T cells were obtained from a haemophilic AIDS patient. The T cells were shown to be infected with HIV, as determined by a reverse transcriptase assay and IFA³. These cells were grown in the absence of PHA for 5 days and then solubilized in SDS. Rabbit polyclonal antisera against HLA class I and HLA class II framework antigens were obtained from Pel-Freez Biologicals. Rabbit antiserum to β_2 -microglobulin was obtained from Dako Corp. In addition, rabbit antiserum was raised against sucrose-purified HIV³. By immunoblotting, this antiserum was shown to recognize six viral proteins separated by SDS-PAGE under conditions of disulphide bond reduction: the core (p24–25), envelope (gp41, gp120), and polymerase (p31, p51, p66) proteins^{24,40}. Immunoblotting was performed as described in Fig. 1.

Table 1 Serum antibody testing in control and patient groups

Controls	Antibodies to	
	HIV	18K Antigen
Heterosexual/ITP	0/10	0/10
Homosexual or Haemophilic:		
HIV-negative	0/17	1/17
HIV-positive	14/14	0/14
Patients		
Homosexual or haemophilic:		
Lymphadenopathy, ITP	16/16	16/16
AIDS	23/23	21/23

The control group consisted of 10 heterosexual subjects with ITP and 31 asymptomatic homosexual men and haemophiliacs. Seventeen of the asymptomatic controls were HIV antibody-negative (10 homosexual, 7 haemophilic) and 14 were HIV antibody-positive (7 homosexual, 7 haemophilic). The patient group consisted of six patients with lymphadenopathy (4 homosexual, 2 haemophilic), 10 patients with ITP (6 homosexual, 4 haemophilic), 16 patients with *Pneumocystis carinii* pneumonia (10 homosexual, 6 haemophilic) and seven patients with Kaposi's sarcoma (all homosexual). Serum antibody against HIV was detected by IFA and immunoblotting^{3,24}; serum antibody against the 18K T-cell protein was detected as described in Fig. 1. None of the 10 heterosexual patients with ITP had serum antibody against the 18K T-cell antigen. Sera from 30 of 31 healthy homosexual and haemophilic controls were negative for antibody against this protein. In contrast, all 16 homosexuals and haemophiliacs with lymphadenopathy or ITP and 21/23 patients with AIDS had serum antibody against 18K antigen.

autoantibody was associated with clinical disease rather than simple exposure to HIV. Antibody against the 18K T-cell antigen was not detected in sera from 40 of 41 heterosexual or 'healthy' homosexual and haemophilic controls, even though 14 of these controls showed evidence of HIV infection (Table 1). The 17

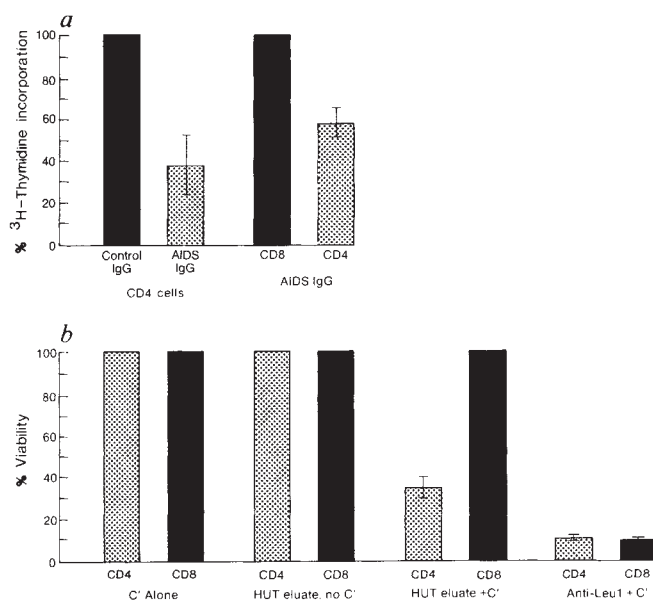


Fig. 4 *a*, Effect of purified IgG on proliferation of PHA-stimulated T cells isolated from peripheral blood. Purified IgG from two homosexual AIDS patients inhibited CD4⁺ cell uptake of ³H-thymidine by a mean of 62% compared to control IgG (range 48–77% inhibition). When the concentration of IgG in the culture medium was decreased, the inhibitory effect of the AIDS samples on cell proliferation was abolished (data not shown). Purified IgG from two other homosexual AIDS patients inhibited CD4⁺ cell proliferation by a mean of 42% compared to proliferation of CD8⁺ cells (range 35–49% inhibition). Uptake of ³H-thymidine by CD8⁺ cells in the presence of purified IgG from the AIDS patients (mean, 5,843 c.p.m.) was comparable to the uptake of ³H-thymidine by CD4⁺ cells in the presence of purified control IgG (mean, 6,100 c.p.m.). *b*, Cytotoxicity of HUT-78 antibody eluates. Incubation of CD4⁺ or CD8⁺ cells with complement alone or with a control HUT eluate in the presence of complement (data not shown) failed to induce cytotoxicity. Incubation with the AIDS-patient eluates in the absence of complement also failed to induce cytotoxicity. Incubation of CD4⁺ cells with the AIDS-patient eluates in the presence of complement induced cytotoxicity in 65% of these cells (range, 60–70%). No cytotoxic effect was seen in the CD8⁺ cells. As a positive control, monoclonal antibody against Leu 1 (CD5; 200 µg ml⁻¹) was shown to be cytotoxic for 90% or more of both CD4⁺ and CD8⁺ cells in the presence of complement. The AIDS eluates had no cytotoxic effect on CD4⁺ cells grown in the absence of PHA (data not shown).

Methods. *a*, T cells were incubated in the presence of purified IgG using a modification of the method of Kiprov *et al.*¹³. Panned Leu-3-positive (CD4⁺) or Leu-2-positive (CD8⁺) T cells (2 × 10⁵ ml⁻¹) were grown in the presence of PHA (4 µg ml⁻¹) and purified IgG (200 µg ml⁻¹ final concentration) for 5 days at 37 °C. Incubations were performed in triplicate using T cells from two normal donors in separate experiments. Cell proliferation was assessed after 5 days by ³H-thymidine incorporation¹³. *b*, Eluates were prepared as described in Fig. 2. The eluates were adjusted for IgG concentration (200 µg ml⁻¹) and added to cultures of PHA-stimulated CD4⁺ or CD8⁺ peripheral-blood T cells in the presence or absence of rabbit complement. Cytotoxicity was assessed by propidium iodide uptake after 24 h using a flow cytometric technique¹⁹. Two separate experiments were performed in triplicate using different T-cell donors, and eluates from two AIDS patients were studied. Seventy per cent of the PHA-stimulated T cells were activated, as assessed by binding of monoclonal anti-HLA-DR antibody to the cultured cells (range, 68–73%).

homosexual controls had normal helper/suppressor T-cell ratios (mean, 1.8). In contrast, 95% of patients with AIDS or AIDS-related conditions were found to have antibody against the 18K T-cell antigen (Table 1). These patients had decreased helper/suppressor ratios (mean, 0.3). Thus the presence of autoantibody against T cells was associated with growth inhibition

and cytotoxicity of lectin-stimulated CD4⁺ cells *in vitro* and with clinical disease and decreased helper/suppressor ratios *in vivo*.

The nature of the 18K T-cell antigen is uncertain. It is distinct from the p17/18 core protein of HIV, because the latter is detected under conditions of disulphide bond reduction on immunoblots. In contrast, the T-cell antigen cannot be detected by immunoblotting after reduction of disulphide bonds (data not shown). We have also found that the T-cell antigen is expressed on HUT-78 cells after infection with murine xenotropic retrovirus. Therefore expression of the antigen does not appear to be specifically induced by HIV infection. Preliminary N-terminal amino-acid-sequence data indicates that the 18K T-cell protein does not share homology with any previously defined polypeptide. Thus the target T-cell protein may be a previously uncharacterized CD4⁺ T-cell activation antigen that is rendered immunogenic by HIV infection.

What is the significance of an autoantibody directed against stimulated CD4⁺ T-cells in AIDS? First, the antibody does not appear to be an epiphenomenon of the disease, because it is found in homosexual men and haemophiliacs with conditions that are considered to be part of the 'pre-AIDS' complex, such as lymphadenopathy and isolated ITP. Thus appearance of the autoantibody precedes the onset of AIDS. Second, induction of this autoantibody may in part explain how patients progress from HIV infection to AIDS. Autoantibody against stimulated T cells may be induced by viral infection, either by interaction of the virus with cell surface components such as lymphocyte CD4 or HLA antigens^{26,27} or by 'molecular mimicry' of cellular proteins, a mechanism that has been postulated in other autoimmune diseases^{28–30}. Once the autoantibody is present, progressive destruction of CD4⁺ T cells may occur on the basis of concomitant T-cell activation and B-cell deregulation^{11,31,32}. Thus an autoimmune response against stimulated T cells may be important in destruction of the immune system in AIDS. Third, recognition of autoantibody production against stimulated T cells may lead to new directions in AIDS therapy aimed either at the autoantibody response itself or at inhibiting expression of the target antigen on T cells. For example, cyclosporin A inhibits the expression of at least two T-cell surface antigens^{33,34}. This inhibition probably constitutes an important immunomodulatory effect of the drug, although its mechanism of action remains undefined³⁴.

In summary, patients with AIDS and AIDS-related conditions produce a cytotoxic autoantibody reactive with lectin-stimulated or HIV-infected T cells of the CD4⁺ phenotype. The role of this antibody in immune deficiency, the relationship of the antibody to viral infection in AIDS, and the nature of the target T-cell antigen are currently under investigation.

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1. Fauci, A. S. *et al. Ann. int. Med.* **100**, 92–106 (1984).
2. Ammann, A. J. *et al. Clin. Immun. Immunopath.* **27**, 315–325 (1983).
3. Levy, J. A. *et al. Science* **225**, 840–842 (1984).
4. Barré-Sinoussi, F. *et al. Science* **220**, 868–871 (1983).
5. Gallo, R. *et al. Science* **224**, 500–502 (1984).
6. Quinnan, G. V. *et al. Ann. int. Med.* **103**, 710–714 (1985).
7. Stricker, R. B., Abrams, D. I., Corash, L. & Shuman, M. A. *N. Eng. J. Med.* **313**, 1375–1380 (1985).
8. Bloom, E. J., Abrams, D. I. & Rodgers, G. M. **256**, 491–493 (1986).
9. Cohen, A. J., Phillips, T. M. & Kessler, C. M. *Ann. int. Med.* **104**, 175–180 (1986).
10. Toy, P. T. C. Y., Reid, M. E. & Burns, M. *Am. J. Hematol.* **19**, 145–150 (1985).
11. Lane, H. C., Masur, H., Edgar, L. C., Whalen, G. & Fauci, A. S. *N. Eng. J. Med.* **309**, 453–459 (1983).

12. Dalakas, M. C., Pezeshkpour, G. H., Gravell, M. & Sever, J. L. *J. Am. Med. Ass.* **26**, 2381-2383 (1986).
13. Kiprov, D. D. *et al. Acquired Immune Deficiency Syndrome*, (eds Gottlieb, M. S. & Groopman, J. E.) 299-308 (New York, 1984).
14. Kloster, B. E., Tomar, R. H. & Spira, T. J. **30**, 330-335 *Clin. Immun. Immunopath.* **30**, 330-335 (1984).
15. Pruzanski, E., Jacobs, H. & Laing, L. P. *AIDS Res.* **1**, 211-220 (1984).
16. Williams, R. C., Masur, H. & Spira, T. J. *J. clin. Immun.* **4**, 118-123 (1984).
17. Tomar, R. H., John, P. A., Hennig, A. K. & Kloster, B. *Clin. Immun. Immunopath.* **37**, 37-47 (1985).
18. Dorsett, B., Cronin, W., Chuma, V. & Iochaim, H. *Am. J. Med.* **78**, 621-626 (1985).
19. Stites, D. P. *et al. Clin. Immun. Immunopath.* **38**, 161-177 (1986).
20. Belisto, D. V., Sanchez, M. R., Baer, R. L., Valentine, F. & Thorbaeck, G. J. *New Engl. J. Med.* **310**, 1279-1282 (1984).
21. Andrieu, J. M., Even, P. & Venet, A. *AIDS Res.* **2**, 163-174 (1986).
22. Ziegler, J. L. & Stites, D. P. *Clin. Immun. Immunopath.* **41**, 305-314 (1986).
23. Spencer, J., Pugh, S. & Isaacson, P. G. *Lancet* **ii**, 983 (1986).
24. Carlson, J. R. *et al. J. Am. Med. Ass.* **253**, 3405-3408 (1985).
25. Selik, R. M., Haverkos, H. W. & Curran, J. W. *Am. J. Med.* **76**, 493-500 (1984).
26. Mann, D. L. *et al. J. Immun.* **131**, 2021-2024 (1983).
27. Klatzmann, D. & Montagnier, L. *Nature* **319**, 10-11 (1986).
28. Billings, P. B., Hoch, S. O., White, P. J., Carson, D. A. & Vaughan, J. H. *Proc. natn. Acad. Sci. U.S.A.* **80**, 7104-7108 (1983).
29. Fujinami, R. S., Oldstone, M. B. A., Wroblewska, Z., Frankel, M. E. & Koprowski, H. *Proc. natn. Acad. Sci. U.S.A.* **80**, 2346-2350 (1983).
30. Walker, E. J., & Jeffrey, P. D. *Lancet* **ii**, 605-607 (1986).
31. Wachter, H. *et al. Lancet* **i**, 97 (1986).
32. Yarchoan, R., Redfield, R. R. & Broder, S. *J. clin. Invest.* **78**, 439-447 (1986).
33. Groenewegen, G., Buurman, W. A., Jeunhomme, G. M. A. A. & van der Linden, C. J. *Transplantation* **40**, 21-25 (1985).
34. Gauchat, J. F., Khandjian, E. W. & Weil, R. *Proc. natn. Acad. Sci. U.S.A.* **83**, 6430-6434 (1986).
35. Andersson, L. C., & Gahmberg, C. G. *Blood* **52**, 57-67 (1978).
36. Stricker, R. B., Wong, D., Saks, R., Corash, L. & Shuman, M. A. *J. clin. Invest.* **76**, 1274-1278 (1985).
37. Engelman, E. G., Benike, F. C., Grumet, F. C. & Evans, R. J. *J. Immun.* **127**, 2124-2128 (1981).
38. Falus, A. *et al. Clin. exp. Immun.* **47**, 103-109 (1982).
39. Stites, D. P., Stobo, J. D. & Wells, J. V. (eds) *Basic and Clinical Immunology* (Appleton and Lange, Los Altos, 1987).
40. Levy, J. A. *et al. Ann. int. Med.* **103**, 694-699 (1985).

Identification of the T-cell and Ia contact residues of a T-cell antigenic epitope

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The precise molecular structure of the antigenic determinant recognized by the T-cell receptor of the CD4-positive cell has not been completely resolved. A major advance in our understanding of this issue has been made by our demonstration of a direct association between an immunogenic peptide and a purified Ia molecule¹. The most likely and economical hypothesis is that antigen binds directly to an Ia molecule creating the antigenic determinant and that this antigen-Ia complex is recognized by the T-cell receptor. We examined in detail a determinant of hen egg-white lysozyme (HEL) contained in the tryptic fragment HEL(46-61), recognized by T cells in H-2^k strains of mice^{2,3}. This peptide binds with a K^d of $\sim 3 \mu\text{M}$ to I-A^k molecules¹. We have already ascertained that (1) the 10-mer HEL(52-61) is the shortest stimulatory peptide⁴; (2) the Leu⁵⁶ residue, the only residue different from mouse lysozyme, is responsible for the immunogenicity⁴; (3) the Leu⁵⁶ and Tyr⁵³ residues are critical for recognition by the T-cell receptor⁵ and (4) HEL(46-61) generates multiple determinants when it associated with the I-A^k molecule^{4,6}. If antigen and Ia interact, the antigen must have two features: it must bind to an Ia molecule and also interact with the T-cell receptor^{7,8}. The two sites do not appear to be laterally separable in this peptide⁵ and are therefore probably composed of distinct but interspersed amino-acid residues. We have now identified the

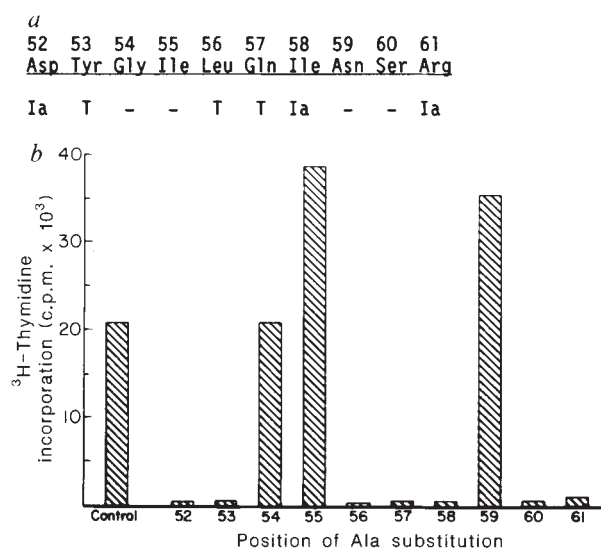


Fig. 1 *a*, The sequence of HEL(52-61) using the standard three-letter code. Numbers refer to the positions in the native HEL molecule. Ia denotes an Ia contact residue; T denotes a T-cell contact residue and a dash a spacer residue. *b*, The ability of Ala substituted peptides to stimulate 2A11 T cells. Various concentrations of peptides containing single Ala substitutions at the indicated positions were tested for their ability to stimulate the 2A11 T cells. The values shown are for the highest concentration of the peptides tested (10 μM). Control refers to the native HEL(52-61) peptide. The potencies of each of the peptides relative to that of the native HEL(52-61) (=1.0) as determined by the concentration required for 50% stimulation are: for the Ala substitutions at 52, 53, 56, 57, 58, 60 and 61 <0.00032; for Ala⁵⁴HEL(52-61), 0.01; for Ala⁵⁵HEL(52-61), 0.316; for Ala⁵⁹HEL(52-61), 0.316. The 2A11 cell line was chosen for these studies because it responds to the lowest concentrations of the 10-mer HEL(52-61) of our panel of reactive T-cell hybrids. In most of our studies we were unable to test peptide concentrations higher than 10 μM because of solubility problems. Thus when a peptide did not stimulate at a concentration of 10 μM we could only conclude that the peptide being examined was at least 3,160-fold less potent than the native HEL(52-61) peptide. **Methods.** The assay used to measure the stimulation of 2A11 or 3A9 T-cell hybridomas has been previously detailed¹⁻⁵. Briefly, 10⁵ peritoneal macrophages from *Listeria monocytogenes*-infected CBA/J mice (~75% express Ia antigens) were adhered to 96-well microtitre plates and fixed with 1% paraformaldehyde; various concentrations of synthetic peptides to be tested were then added along with 2A11 T cells (10⁵). The level of T-cell stimulation was determined by measuring the level of IL-2 in an 18-20 h supernatant using a ³H-thymidine incorporation assay (CTLL cells). The maximal amount of IL-2 released by the T cells in a 100- μl aliquot was generally on the linear portion of the dose-response curve as determined by assessing various dilutions of supernatant. Comparisons between various peptides were always performed by testing a wide concentration range and comparing the concentrations which resulted in half-maximal stimulation. Peptides prepared using the general solid-phase method of Merrifield *et al.*⁴ and purified by HPLC on a Vydac C₁₈ column using a linear gradient of acetonitrile in 0.1% TFA from 0 to 80%. Peptides were quantitated by amino acid analysis and stored at -20 °C until use.

three residues of HEL(52-61) that contact the T-cell receptor and three other residues that contact the I-A^k molecule. From modelling studies we also propose that HEL(52-61) assumes an α -helical conformation as it is bound to I-A^k and recognized by the T-cell receptor.

We examined substituted peptides based on the HEL(52-61) sequence by testing for their ability to stimulate a T-cell hybridoma and by competing for the presentation of the stimulatory HEL(46-61) peptide. A peptide containing a single substitution that is still stimulatory indicates that the substituted residue is not essential. We consider these residues as spacer residues

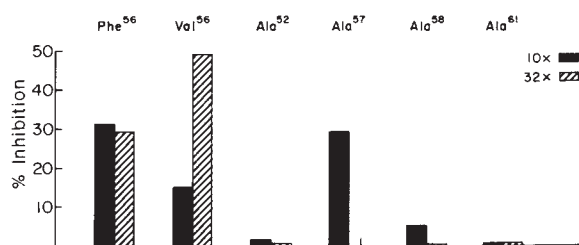


Fig. 2 Inhibition of presentation of HEL(46-61). To a monolayer of fixed macrophages, 0.316 μ M or 1.0 μ M of HEL(46-61) was added along with either medium alone or medium containing 10 μ M of the peptides which have the single substitution shown of HEL(52-61). 2A11 T cells were added and their stimulation was determined using the standard procedure. The percent inhibition was determined as follows: % inhibition = [(c.p.m. of medium control) - (c.p.m. with inhibitor)/(c.p.m. of medium control)] $\times$ 100. The value of the c.p.m. of the medium control was 56,035 for 1.0 μ M HEL(46-61) and 53,326 for 0.316 μ M.

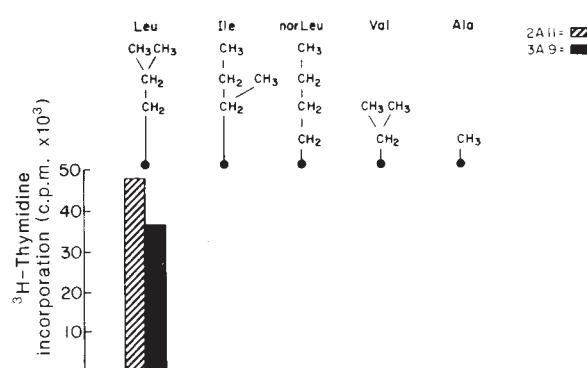


Fig. 3 The ability of substituted peptides at position 56 to stimulate 2A11 or 3A9 cells. Peptides containing the single amino-acid substitution shown at position 56 of HEL(52-61) were tested for their ability to stimulate either 2A11 or 3A9 cells. The values shown are for the highest concentration tested (10 μ M) and represent the mean of triplicate values. The structure of the side chains of each of these substitutions is shown.

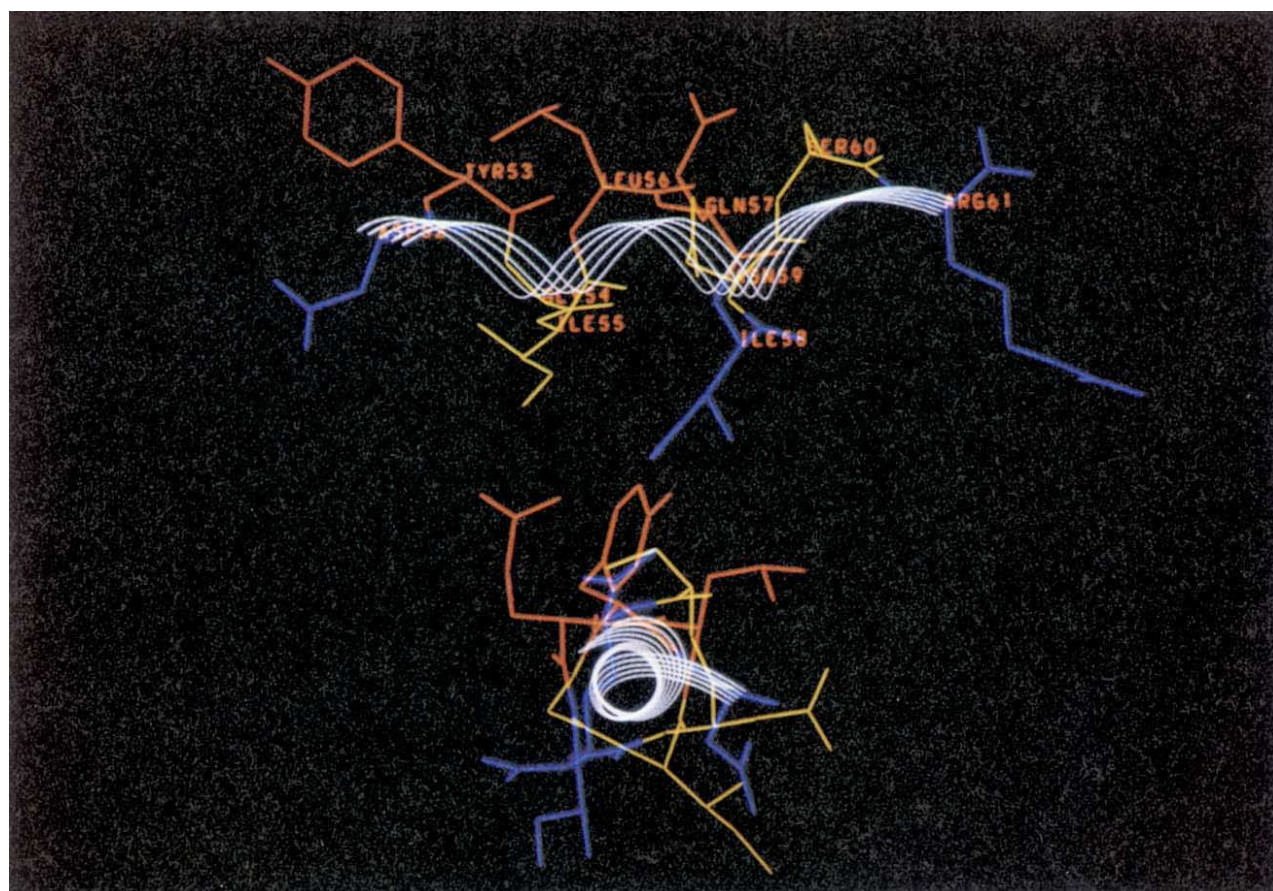


Fig. 4 Modelling the conformation of HEL(52-61). The T-cell and Ia contact residues were segregated by placing the peptide HEL(52-61) into an idealized α -helix and minimizing the structure. The top view is from the side of the α -helix with the amino terminus on the left and the backbone of the helix is traced by the white ribbon. The T-cell contact residues are shown in red, the Ia contact residues in blue and the spacer residues in yellow. The bottom view represents a 90° rotation of the top view and looks down the axis of the helix. The macromolecular modelling system MENDYLtm (Tripos. Assoc. Inc) was used to build, minimize, and view the structures interactively in three dimensions. The calculations were performed on a MicroVax IItm and displayed on an Evans and Sutherland PS330tm. The molecular mechanics method employed was Kollman's (AMBER)²⁰ force field with a modified potential function for out-of-plane bending. (Tripos. Inc) In all cases, the two-stage minimization option was used to reduce the possibility of discontinuities in the surface. The first stage uses the Simplex method which was keyed to atoms which had a force of greater than 2.5 millidynes²¹. The second stage uses the conjugate gradient method. The first derivatives are calculated analytically, then all the atoms simultaneously move down their gradients. We used a distance-dependent dielectric and a residue-based, nonbonded-interaction cutoff distance of 8 Å and the nonbonded list was updated every five iterations. The scaling factor for the 1-4 interactions (both van der Waals and electrostatic) were set to 0.5 as per Kollman's recommendation and the overall convergence factor was set to 0.05 Δ kcal per mol²⁰.

and by it imply that their specific side chain is not crucial for the interactions, but that some amino acid must be in this position to allow for the formation of the correct conformation.

A peptide containing a single substitution which is not stimulatory indicates that the residue in the substituted position may be essential for contacting either the T-cell receptor or the Ia molecule. It is also possible that the substitution may result in a conformational change in the peptide which indirectly affects its interaction with either of them. Such a non-stimulatory peptide when tested in a competition assay can either compete, implying that the peptide still binds to I-A^k; thus the substituted residue is not critical for binding to Ia and should be involved directly or indirectly in contacting the T-cell receptor; or it can fail to compete, which means that the peptide does not bind to I-A^k. The substituted residue must therefore be involved in contacting the Ia molecule. We classify the former as 'T-cell contact residues'⁵ and the latter as Ia contact residues⁵.

We tested ten substituted peptides derived from the shortest stimulatory peptide HEL(52-61). Each contained a single Ala substitution at one of the ten positions. These peptides examined the contribution of each of the amino acid side chains by replacing them with the simplest side chain which still had chirality, that of Ala. Only three of the Ala substituted peptides stimulated the 2A11 cells, those being Ala⁵⁴HEL(52-61), Ala⁵⁵HEL(52-61), and Ala⁵⁹HEL(52-61) (Fig. 1b). Gly⁵⁴, Ile⁵⁵ and Asn⁵⁹ were classified therefore as spacer residues (Fig. 1a). Ser⁶⁰ was also a spacer residue in that it could be deleted without affecting the ability of a peptide to stimulate.

To examine the other positions we used the Ala-substituted peptides and tested them in our functional competition assay. Using this assay, we previously identified the Tyr⁵³ and Leu⁵⁶ as T-cell contact residues⁵. Confirming our previous results the Ala⁵³HEL(51-61) competed for the presentation of HEL(52-61) (Fig. 2). When the other 4 peptides were examined only one, that with an Ala at position 57, competed. This indicates that the Gln⁵⁷, in addition to the Tyr⁵³ and Leu⁵⁶, is a T-cell contact residue. Conversely, the inability of the Ala⁵²HEL(52-61), Ala⁵⁸HEL(52-61) and Ala⁶¹HEL(52-61) to compete indicates that Asp⁵², Ile⁵⁸ and Arg⁶¹ are the Ia contact sites (Fig. 1a).

We examined the specificity of the recognition of two of the putative T-cell contact residues, Tyr⁵³ and Leu⁵⁶, by testing a series of substituted peptides. These results shown in Fig. 3 demonstrated a high degree of specificity in the recognition of the Leu⁵⁶ side chain, consistent with the immunoglobulin nature of the T-cell receptor⁹. A slightly different pattern emerged when the specificity of recognition of the Tyr⁵³ side chain was examined (not shown). The 2A11 T cells absolutely required the native Tyr residue and would not respond to any derivatives, including 3-NO₂-Tyr⁵³ and Phe⁵³. Conversely, the 3A9 cell line responded to them.

Using the macromolecular modelling system MENDYL we modelled the conformation of the bound peptide. Two of the T-cell contact residues, Tyr⁵³ and Leu⁵⁶, are located 3 residues apart. In an idealized α -helix there are 3.6 residues per turn; thus, in an α -helix the Tyr⁵³ and Leu⁵⁶ side chains would be oriented on the same face of the peptide. We considered the HEL(52-61) peptide as an α -helix and minimized the structure to obtain the conformation shown in Fig. 4. We observed a segregation of the residues that contact the T-cell receptor from those that contact the Ia molecule. The Tyr⁵³, Leu⁵⁶, and Gln⁵⁷ side chains are all oriented in one direction (coloured red), whereas the Asp⁵², Ile⁵⁸ and Arg⁶¹ side chains are oriented in an opposite direction (coloured blue). This orientation of the side chains would allow one face of the peptide to bind to the Ia molecule, and the opposite face to contact the T-cell receptor. There have been several other studies which implicate an α -helical conformation in the antigen recognized by T cells¹⁰⁻¹². The peptide HEL(52-61) in the native structure of HEL is located in a series of β -pleated sheets¹³, whereas in solution HEL(52-61) has no detectable conformation. The slow associ-

ation rate of a peptide with Ia¹⁴ may reflect a process such as the folding of HEL(52-61) into an α -helix. Despite the possibility of other conformers, we favour the α -helical conformation for three reasons. First, the α -helical form allowed the most complete segregation of the T-cell and Ia contact residues and second, it has the lowest enthalpy of the conformers we examined. Third, we have preliminary results that a homologue of HEL(52-61) which has a high α -helical propensity due to a stabilization of the helix dipole is 300-fold more stimulatory than the HEL(52-61) peptide.

Having multiple ways of forming an Ia-binding site is essential for our current model of the formation of a T-cell antigenic determinant. A *sine qua non* for the stimulation of T cells may be that the antigen must first interact with an Ia molecule. This places a tremendous burden upon the Ia molecule to be able to bind at least one determinant from every potential antigen. Having multiple ways to form possible binding modes would allow many different antigens to be recognized by T cells. The limited data on the Ia contact residues of the defined T-cell determinants makes it currently impossible to identify a canonical Ia-binding structure¹⁵⁻¹⁷. It has recently been reported that the association of peptides with Ia can be explained by their sequence homologies to the Ia molecule, based on studies of an immunogenic peptide from the λ repressor molecule¹⁸. This result cannot be generalized inasmuch as none of four peptides examined by us have any significant homology to I-A^k.

It now appears that there is a single site on the Ia molecule which binds peptides. The studies of Buus *et al.*¹⁹ indicate that peptides restricted by the same allele of the Ia molecule can compete for the binding to that Ia molecule. We have analysed three other I-A^k-restricted peptides, HEL(34-45), RNase (41-55), and RNase (105-124), and found that they have no sequence homology (R. G. Lorenz and P.M.A., unpublished data). But they all bind to the same site on the I-A^k molecule because they compete for binding with HEL(46-61). We are left with the conclusion that many unrelated peptides bind to a single Ia site and that the Ia molecule has a broad binding specificity. Finally, while the specificity of the Ia molecule for peptides is broad, the resultant effect of such interaction on the peptide is to create a unique structure. The Ia may impose on the peptide a stable secondary structure whereupon the side chains of some residues—the T-cell residues identified herein—become accessible for recognition along with portions of the Ia molecule, to the T-cell receptor.

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- Babbitt, B., Allen, P. M., Matsueda, G., Haber, E. & Unanue, E. R. *Nature* **317**, 359-361 (1985).
- Allen, P. M. & Unanue, E. R. *J. Immun.* **132**, 1077-1079 (1984).
- Allen, P. M., Strydom, D. J. & Unanue, E. R. *Proc. natn. Acad. Sci. U.S.A.* **81**, 2489-2493 (1984).
- Allen, P. M., Matsueda, G. R., Haber, E. & Unanue, E. R. *J. Immun.* **135**, 368-373 (1985).
- Babbitt, B. P., Matsueda, G. R., Haber, E., Unanue, E. R. & Allen, P. M. *Proc. natn. Acad. Sci. U.S.A.* **83**, 4509-4513 (1986).
- Allen, P. M., McKean, D. J., Beck, B. N., Sheffield, J. & Glimcher, L. H. *J. exp. Med.* **162**, 1264-1274 (1985).
- Rosenthal, A. S. *Immun. Rev.* **40**, 136-152 (1978).
- Heber-Katz, E., Hansburg, D. & Schwartz, R. H. *J. molec. cell. Immun.* **1**, 3-9 (1983).
- Kronenberg, M., Siu, G., Hood, L. E. & Shastri, N. A. *Rev. Immun.* **4**, 529-591 (1986).
- Pincus, M. R., Gerewitz, F., Schwartz, R. H. & Scheraga, H. *Proc. natn. Acad. Sci. U.S.A.* **80**, 3297-3301 (1983).
- Spouge, J. L. *et al. J. Immun.* **138**, 204-212 (1987).
- Carbone, F. R., Fox, B. S., Schwartz, R. H. & Paterson, Y. *J. Immun.* **138**, 1838-1844 (1987).
- Blake, C. C. *et al. Nature* **206**, 757-760 (1965).
- Buus, S., Sette, A., Colon, S. M., Jenis, D. M. & Grey, H. M. *Cell* **47**, 1071-1077 (1986).
- Hansburg, D., Heber-Katz, E., Fairvill, T. & Apella, T. *J. exp. Med.* **158**, 25-39 (1983).
- Watts, T. H., Gariepy, J., Schoolnik, G. K. & McConnell, H. M. *Proc. natn. Acad. Sci. U.S.A.* **82**, 5480-5484 (1985).
- Berkower, I. J., Buckenmeyer, G. K. & Berzofsky, J. A. *J. Immun.* **136**, 2498-2503 (1986).
- Güillet, J.-G. *et al. Science* **235**, 865-870 (1987).
- Buus, S., Sette, A., Colon, S. M., Miles, C. & Grey, H. M. *Science* **235**, 1353-1358 (1987).
- Weiner, S. J., Kollman, P. A., Nguyen, D. T. & Case, D. A. *J. comput. Chem.* **7**, 230-252 (1986).
- Nader, J. A. & Mead, R. *Comp. J.* **7**, 308-313 (1965).

Site-specific mutagenesis of AIDS virus reverse transcriptase

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Human immunodeficiency virus (HIV) is the causative agent of AIDS (acquired immune deficiency syndrome)¹⁻³ a disease which poses a serious challenge to modern medicine. If we are to conquer this disease we will need a protective vaccine or effective drugs able to block the life cycle of the virus. An early stage in the invasion of the host cell is the conversion of the RNA genome of the virus to a double-stranded DNA intermediate which subsequently becomes integrated into the host cell chromosome⁴. The enzyme reverse transcriptase is crucial in this process and is thus an obvious chemotherapeutic target. In this study we have used site-directed mutagenesis of this enzyme expressed in *Escherichia coli* to reveal several important functional regions of the protein including putative components of the triphosphate binding site and pyrophosphate exchange sites.

As a first step the HIV reverse transcriptase was expressed to a high level as a polypeptide of relative molecular mass 66,000 (*M*_r 66K) using an M13 vector (details of this expression system and the RT construct mpRT4 will be published elsewhere⁵). Briefly, the polypeptide expressed corresponds to the native RT

polypeptide as defined previously^{6,7} with the addition of two amino-acid residues (Asn, Ser) at the N terminus.

Our objective was to probe the functional regions of the gene using site-directed mutagenesis. We decided to focus on regions which are similar in related enzymes, working on the basis that conservation of sequence is likely to reflect the need to maintain function. We made extensive use in this analysis of earlier comparative studies made at the level of predicted amino acid sequence⁶⁻⁹. From this analysis we identified six regions (Fig. 1) as prime targets for mutagenesis. All six showed striking similarity to corresponding regions in other reverse transcriptases⁶ and in addition region E contained a sequence motif which is very common amongst RNA polymerases^{6,9}. The characteristic feature of this motif is the sequence Asp-Asp embedded in a short region made up predominantly of non-polar amino acids. The function of this region is unknown but it has been suggested that it may be important in template binding⁹.

Site-directed mutagenesis using oligonucleotides was used to create the mutants shown in Fig. 1. Each mutation either introduced a new restriction site into the gene or deleted an existing one, and so these changes were used as markers to identify the mutants. Each mutation was also designed to introduce a single amino-acid substitution and so encode a polypeptide identical in size to the parent. The polypeptides induced by the mutants were therefore analysed by polyacrylamide gel electrophoreses (Fig. 2). Small changes in the mobility of the mutant polypeptides could probably be explained by the nature of the amino-acid substitutions introduced. The amounts of enzyme expressed by the mutants and responses to inhibition by phosphonoformic acid (PFA) or azidothymidine-triphosphate (AZT-TP) are summarised in Table 1.

Fig. 1 Position of mutations and conserved regions in the HIV RT. The HIV reverse transcriptase (RT) polypeptide is represented as a linear molecule with amino-acid residue numbers based on the sequence of the LAV strain of HIV and conserved regions (A-F) shown as solid bands, with (expanded) sequences in single-letter code for amino acids. These were identified as they have previously been shown to share amino-acid sequences similar to those of other retroviral *pol* genes⁶⁻⁸, and in the case of region E (residues 180-190) there is also homology with a number of non-retroviral polymerases⁹. Regions in which single amino-acid substitutions have been introduced (Table 1) are shown above the reverse transcriptase polypeptide with the amino-acid changes illustrated. Two other identified conserved regions are shown below the reverse transcriptase polypeptide. The putative location of the RNase H activity associated with the reverse transcriptase (ref. 6) is depicted at the carboxy-terminal of the polypeptide (shaded area).

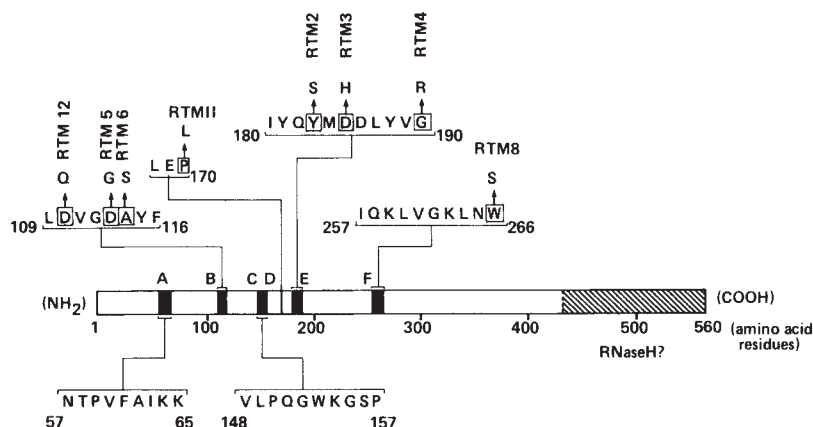


Table 1 Reverse transcriptase activity induced by mutants obtained by site-directed mutagenesis

M13 construct	RT activity (% mpRT4)	Apparent <i>M_r</i> of RT polypeptide ($\times 10^{-3}$)	ID ₅₀ (μ M)	
			PFA	AZT-TP
rnprT4 (wild-type RT)	100	66	0.5	0.06
rnptac18 (No RT insert)	<0.01	—	—	—
RTM2 (Tyr → Ser, 183)	1.2	67	—	—
RTM3 (Asp → His, 185)	<0.01	65	—	—
RTM4 (Gly → Arg, 190)	23	65	1.1	0.18
RTM5 (Asp → Gly, 113)	59	65	2.5	1.0
RTM6 (Ala → Ser, 114)	80	64	3.2	0.56
RTM8 (Tyr → Ser, 266)	43	66	1.0	0.79
RTM11 (Pro → Leu, 170)	100	65	0.4	0.08
RTM12 (Asp → Glu, 110)	<0.01	65	—	—

Reverse transcriptase (RT) activity was assayed using poly(rA)-oligo(dT) as primer template in bacterial extracts by standard methods⁵. The 50% inhibition values (ID₅₀) were obtained directly from plots of reverse transcriptase activity against log₁₀ (inhibitor concentration). All 'RT' mutants were made by site-directed mutagenesis¹³ using synthetic oligonucleotides (synthesised on a DuPont Coder 300 machine) of between 17 and 21 nucleotides in length, introducing either a single or double nucleotide substitution.

One of the most interesting regions identified by sequence comparison was region E. Three independent mutations were introduced into this sequence. The mutation having the most dramatic effect was Asp→His at residue 185 in mutant RTM3. This substitution, which disrupted the Asp-Asp doublet, completely destroyed the reverse transcriptase activity of the protein. Two other changes in this region (Tyr→Ser at residue 183 in RTM2, and Gly→Arg at residue 190 in RTM4) resulted in reduced enzyme activity. Mutant RTM2 expressed very low levels of activity and so was not examined further. The enzyme from RTM4 was tested for inhibition by PFA or AZT-TP but resembled the parent in sensitivity. Tyr 183 is well conserved amongst the reverse transcriptases and so it is not difficult to understand that a substitution at this position might be disruptive. However Gly 190 is not well conserved and so the effects of substitution of this residue are less easily explained. It may be that the reduction in the overall hydrophobicity of the region caused by the introduction of an arginine residue is sufficient to impair function at the site. Further biochemical analysis of these enzymes should help to define the function of this region.

A feature of region B is that, like region E, it contains two aspartate residues, although in this case they are not contiguous. Two of the mutations introduced in this region were designed to remove one or other. In one case (Asp→Glu at residue 110 in RTM12) the alteration resulted in complete loss of enzyme activity, whereas in the other (Asp→Gly at residue 113 in RTM5) there was a significant reduction, resulting in induction of about 60% of the activity observed with mpRT4.

Amino-acid substitutions in two regions (region F, Trp→Ser at residue 266 in RTM8, region B, Asp→Gly at residue 113 in RTM5 and Ala→Ser at residue 114 in RTM6), rendered the enzyme less sensitive to inhibition by AZT-TP. Thus both regions are good candidates for components of a triphosphate binding site. However, in addition to changing the sensitivity of RT to AZT-TP, the mutations in RTM5 and RTM6 both had significant

effects on the sensitivity of the enzyme to PFA inhibition. Because PFA is a known pyrophosphate analogue this appears to imply an additional function for region B in pyrophosphate exchange. Not all conserved amino acids seem to be essential for enzyme function because Pro→Leu in RTM11 had no apparent effect on the biochemical properties of the enzyme.

It is clear from these studies that subtle changes in the reverse transcriptase polypeptide can result in an apparent reduction in binding affinity for the antiviral agents PFA (mutants RTM5 and RTM6), and AZT-TP (mutants RTM5, RTM6 and RTM8). This observation is not surprising as we have previously shown that single amino-acid substitutions in the herpes-simplex-virus-encoded DNA polymerase can also result in decreased sensitivity to a number of polymerase inhibitors¹⁰. Note that in the case of the herpes enzyme, the *pol* mutations led to attenuation in pathogenicity of the mutant viruses^{11,12}. We do not know what effects the mutations in HIV reverse transcriptase described here would have if present in the virus itself, but drawing an analogy with our experience with herpes mutants it would seem likely that such mutations would be detrimental to virus growth. We are currently testing this possibility by inserting the mutant reverse transcriptase genes back into infectious virus.

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1. Barré-Sinoussi, F. *et al. Science* **220**, 868-871 (1983).
2. Levy, J. A. *et al. Science* **225**, 840-842 (1984).
3. Popovic, M., Sarngadharan, M. G., Read, E. & Gallo, R. C. *Science* **224**, 497-500 (1984).
4. Temin, H. M. & Baltimore, D. *Adv. Virus Res.* **17**, 129-186 (1972).
5. Larder, B. A., Purifoy, D. J. M., Powell, K. L. & Darby, G. *EMBO J.* (submitted).
6. Johnson, M. S. *et al. Proc. natn. Acad. Sci. U.S.A.* **83**, 7648-7652 (1986).
7. Toh, H., Hayashida, H. & Miyata, T. *Nature* **305**, 827-829 (1983).
8. Patarca, R. & Haseltine, W. A. *Nature* **309**, 728 (1984).
9. Kamar, G. & Argos, P. *Nucleic Acids Res.* **12**, 7269-7282 (1984).
10. Larder, B. A., Kemp, S. D. & Darby, G. *EMBO J.* **6**, 169-175 (1987).
11. Larder, B. A. & Darby, G. *Virology* **146**, 262-271 (1985).
12. Larder, B. A., Lisle, J. J. & Darby, G. *J. gen. Virol.* **67**, 2501-2506 (1986).
13. Zoller, M. J. & Smith, M. *Nucleic Acids Res.* **10**, 6487-6500 (1982).

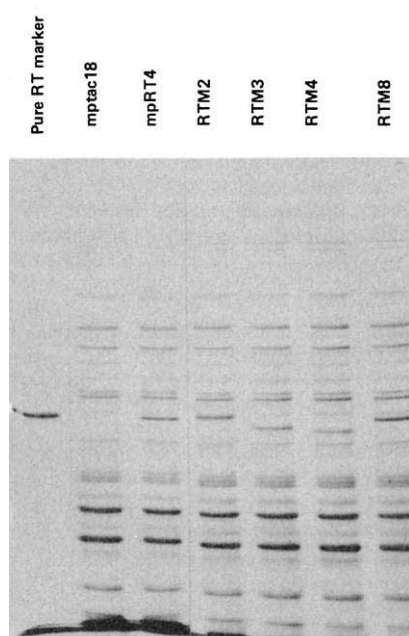


Fig. 2 Electrophoretic behaviour of mutant reverse transcriptase polypeptides. To assess the amount of reverse transcriptase polypeptide present (especially when no activity was detectable), each mutant-infected cell preparation was extracted and subjected to electrophoresis on a 7.5% polyacrylamide gel which was then stained with Coomassie Brilliant blue. The reverse transcriptase polypeptide was obvious as a polypeptide not present in *E. coli* infected with mptac18. Selected mutants are shown and all mutant enzymes were examined. The M_r of each polypeptide was calculated and is indicated in Table 1.

The glycoprotein encoded by the X-linked chronic granulomatous disease locus is a component of the neutrophil cytochrome *b* complex

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The bacteriocidal capacity of phagocytic cells is impaired in X-linked chronic granulomatous disease (X-CGD), a disorder characterized by the absence of functional plasma-membrane-associated NADPH oxidase¹. The components of this oxidase system, their correspondence with specific genetic loci, and the primary protein defect in X-CGD remain incompletely defined. We recently reported cloning of the putative X-CGD gene on the basis of DNA linkage^{2,3}. To identify the predicted protein *in vivo*, antibodies were raised to a synthetic peptide derived from the complementary DNA sequence and to a fusion protein produced in *Escherichia coli*. In Western blots antisera detect a neutrophil protein of relative molecular mass in 90,000 (90K) that is absent in X-CGD

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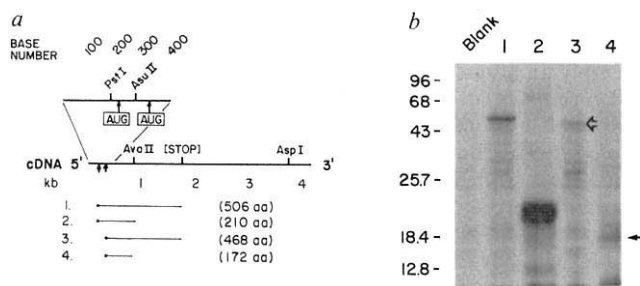


Fig. 1 Translation of *in vitro* synthesized RNAs. *a*, cDNA constructions used to generate RNAs and the polypeptide products anticipated from initiation of translation at the indicated AUGs. The full-length X-CGD restriction map is indicated in the middle with the two in-frame AUGs indicated by upward arrows. Above, an expanded version of the 5'-portion of the cDNA is depicted. Base numbers correspond to those in our previous cDNA sequence³. *b*, Products of *in vitro* translations. Blank indicates no addition of RNA. Lane 1: RNA synthesized from full-length *AspI* plasmid containing both AUGs. Lane 2: *AvaII*-digested plasmid; Lane 3: RNA synthesized from 5'-truncated *AspI* linearized plasmid; Lane 4: *AvaII*-digested 5'-truncated plasmid. Open and closed arrows, respectively indicate the positions of the polypeptides derived from the 5'-truncated constructions. For synthesis of RNA containing the entire coding region, including both AUGs, the 3.5 kb *PstI*-*AspI* fragment of bacteriophage 99 (ref. 3) was subcloned into the vector pGem-4 (Promega Biotec). The first AUG was removed from the construction by cleavage with *AsuII*, ligation to synthetic *PstI* linkers, and recloning of the 5'-truncated *PstI*-*AspI* fragment in pGem-4. Plasmids were linearized either with *AspI* (entire coding region) or with *AvaII* (3'-truncated versions) prior to *in vitro* transcription with T7 polymerase using protocols supplied by Promega Biotec. RNA transcription reactions were extracted with phenol, precipitated, and used for *in vitro* translation in wheat germ extracts (Promega Biotec) with ³⁵S-methionine as a radioactive amino acid. Following incubation, translation extracts were treated with RNAase A, precipitated with trichloroacetic acid, washed with acetone, and electrophoresed through 12.5% acrylamide. The acrylamide gel was electroblotted and exposed to X-ray film.

patients. Antisera also react with the larger component of cytochrome *b* recently purified from neutrophil plasma membranes as a complex of glycosylated 90K and non-glycosylated 22K polypeptides⁴. Based on our identification of the X-CGD protein *in vivo*, we propose that one of its critical roles is to interact with the 22K species to form a functional cytochrome *b* complex.

From its cDNA sequence, the X-CGD mRNA encodes a polypeptide of at least 54–58K which contains potential *N*-glycosylation sites and bears no significant homology to known proteins. Of the two potential in-frame translation initiator codons located at nucleotide positions 208–210 and 322–324 in the X-CGD cDNA sequence³, the latter was initially favoured on the basis of consensus rules for selection of functional AUGs⁵. That the AUG at position 208–210 might be used was proposed by Creighton in view of the potential for a cleavable 20 amino-acid signal peptide following immediately thereafter⁵. Kozak suggested that the X-CGD mRNA might be bifunctional⁵. To resolve this issue and provide a more confident estimate of the primary translation product, RNAs synthesized *in vitro* by T7 polymerase transcription of cDNA containing either both AUGs or only that at 322–324 were translated in wheat germ extract⁷ (Fig. 1*a*). When both AUGs were present on full-length RNA, a polypeptide with an apparent molecular mass of 52K was synthesized (Fig. 1*b*). Truncation of the plasmid at an *AvaII* site within the coding region yielded a product of 21K (lane 2). To determine which of the two potential AUGs was used in translation, the 5'-AUG was deleted from the cDNA plasmid by cleavage at a unique *AsuII* site. Full-length or 3'-truncated RNAs containing only the second AUG were translated con-

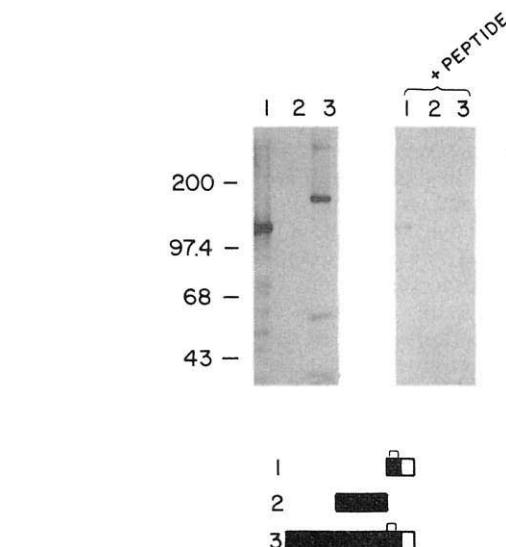


Fig. 2 Western blots of β -galactosidase fusion proteins. Antisera were raised against a synthetic peptide corresponding to 20 amino acids near the C-terminus of the predicted X-CGD protein (CGPEALAEATLSKQSISSNES). The peptide and a peptide-KLH conjugate were prepared by Cambridge Research Biochemicals. Female New Zealand white rabbits were immunized by intramuscular injection with 300 μ g KLH-conjugated peptide emulsified with Freund's complete adjuvant. Booster injections of 250–300 μ g with incomplete Freund's adjuvant were given at 4 and 7 weeks. Sera were obtained 11 days after the second booster injection and affinity-purified using peptide coupled to Affi-gel 10 (Biorad). Antisera reactivity to the peptide was monitored using an enzyme-linked immunoabsorbent assay (ELISA). β -Galactosidase fusion proteins were prepared by cloning different restriction fragments of X-CGD cDNA into the expression vectors Agt11 (ref. 16) or pUR 292 (ref. 17). These X-CGD cDNA fragments are shown schematically in the lower portion of the figure (translated region is shaded); the segment corresponding to the synthetic peptide is bracketed. X-CGD cDNA fragments contained in each construct were as follows: fusion 1, *EcoRI*/*SacI*; fusion 2, *AvaII*/*EcoRI*; fusion 3, *PstI*/*SacI*. Extracts of IPTG-induced *E. coli* containing 0.2–0.5 μ g fusion protein were separated on a 7.5% SDS-polyacrylamide gel¹⁸, and electroblotted onto nitrocellulose¹⁹ with 0.1% SDS in the transfer buffer. The blot was reacted with affinity-purified antibody, and bound antibody detected with an affinity-purified alkaline-phosphatase-conjugated goat antibody to rabbit IgG (Promega Biotec), using the protocol supplied by Promega Biotec, except that nonspecific protein binding to nitrocellulose was blocked with 5% nonfat dry milk. Lanes 1, 2 and 3 are *E. coli* extracts containing the corresponding fusion protein. The incubation with antipeptide antibody was performed with peptide at 10 μ g ml⁻¹ in the blot shown on the right. Size markers, M_r in thousands, are depicted on the left.

siderably less efficiently into shorter polypeptides of 48 and 18K, respectively (lanes 3 and 4). These data demonstrate that the in-frame AUG at position 208–210, although not conforming well to the Kozak consensus sequence⁵, is preferentially used for translation and tend to support the proposal of Creighton⁶ that the primary translation product of X-CGD mRNA is 506 amino acids with a potential cleavable signal peptide of 20 amino acids at the N terminus. An additional canonical *N*-glycosylation site six amino acids preceding the methionine at position 322–324 brings to five the total in the X-CGD sequence. Three of these are clustered in the 75 N-terminal amino acids and before a large putative transmembrane domain (amino acids 83–110 of the predicted protein)³.

To identify the *in vivo* protein product of the X-CGD gene, antibodies were first raised in rabbits to a synthetic peptide conjugated to keyhole limpet haemocyanin (KLH). The 20 amino-acid peptide corresponds to the predicted protein sequence that begins 34 amino acids proximal to the predicted

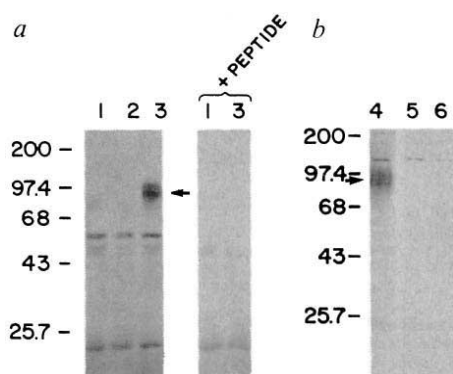


Fig. 3 Western blot of solubilized neutrophils. *a*, Anti-peptide antibody. Lanes 1 and 2, solubilized neutrophils from two unrelated patients with X-CGD. Lane 3, solubilized neutrophils from a normal volunteer. In the blot on the right, incubation with anti-peptide antibody was performed in the presence of peptide at $12.5 \mu\text{g ml}^{-1}$. *b*, Anti-X-CGD fusion protein antisera. Lane 4, normal neutrophils. Lanes 5 and 6, neutrophils from two patients with X-CGD; lane 5 is from the same patient as in lane 1; lane 6 is from a third, unrelated patient. Size markers, M_r in thousands, are depicted on the left. Arrows indicate the position of the $\sim 90\text{K}$ protein detected by the antisera.

Methods. Neutrophils were isolated from peripheral blood²⁰, resuspended in 0.34 M sucrose and incubated with 5 mM diisopropylfluorophosphate for 10–30 min, then solubilized in electrophoresis sample buffer¹⁸ containing 2.5% SDS and 2% β -mercaptoethanol. Samples of 40–50 μg total neutrophil protein²¹ were subjected to electrophoresis on a 10% SDS-polyacrylamide gel, transferred to nitrocellulose, and immunoblotted as described in Fig. 2. Anti-X-CGD fusion protein antiserum was raised against a β -galactosidase fusion protein expressed in *E. coli* that contained the entire X-CGD sequence (fusion 3 in Fig. 2). The fusion protein was isolated by SDS-polyacrylamide electrophoresis of solubilized *E. coli*; the band containing the fusion protein was excised, minced and emulsified in Freund's complete adjuvant (for primary immunization) or incomplete Freund's adjuvant (for booster injections). Female New Zealand white rabbits were immunized by intramuscular injection of 250 μg fusion protein and boosted with 200 μg at 4, 12 and 20 weeks; sera were obtained 7 days later and used at a 1:1000 dilution for the Western blot.

carboxy terminus. The reactivity of the antisera was examined in a series of β -galactosidase fusion proteins expressed in *E. coli* (Fig. 2). These included fusions either containing or lacking the C terminus. *E. coli* extracts containing the hybrid proteins were analysed in Western blots using anti-peptide antibody (Fig. 2). Antibody reacted with the fusion proteins containing the entire X-CGD protein sequence or the C-terminal 51 amino acids, but not with the fusion protein that included a central region of the polypeptide. Antibody specificity is shown by competition with excess peptide. These data demonstrate in-frame translation of the X-CGD sequence fused to β -galactosidase and specificity of antisera for protein synthesized in *E. coli* carrying the appropriate segment.

Extracts of neutrophils were analysed in a similar fashion to identify the protein *in vivo*. As shown in Fig. 3, a broad band migrating at approximately 90K was detected in normal neutrophils, and was absent in neutrophils obtained from three unrelated patients with classic X-CGD. Two of these patients were previously shown to lack X-CGD mRNA in cultured monocytes³. The broad appearance of the band is secondary to extensive glycosylation (see below). The detection of the 90K species, as well as a cross-reacting protein of approximately 60K, was inhibited by peptide. The correspondence of only the 90K protein to the product of the X-CGD gene is demonstrated by its specific absence in X-CGD neutrophils. The 60K species was present in both normal and X-CGD neutrophils and reacted with sera obtained only from one rabbit; sera from other immunized rabbits each detected several different cross-reacting pro-

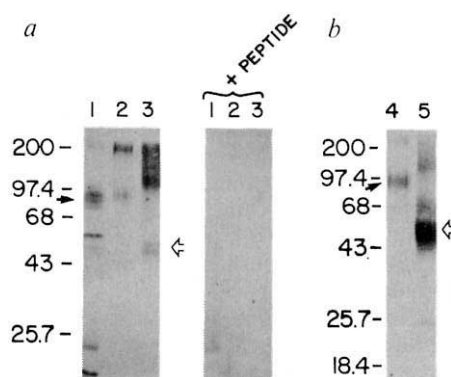


Fig. 4 Western blot of solubilized neutrophils and purified cytochrome *b*. Solubilized neutrophils were prepared from a normal volunteer as described in Fig. 3. Purification of cytochrome *b* from neutrophil plasma membranes and treatment with endoglycosidase F was as described⁴; two separate preparations are shown, *a* and *b*. Western blots were performed as described in Figs 2 and 3, using a 10% SDS-polyacrylamide gel (*a*) and a 7–18% gradient gel (*b*) for protein separation. *a*, Anti-peptide antisera. Lane 1, normal neutrophils (40 μg protein). Lane 2, purified cytochrome *b* (0.5 μg protein). Lane 3, purified cytochrome *b* digested with endoglycosidase (0.5 μg protein). Competition with peptide was performed at 10 μg peptide per ml. *b*, Anti-X-CGD fusion protein antisera. Lane 4, purified cytochrome *b* (0.75 μg protein). Lane 5, purified cytochrome *b* digested with endoglycosidase. Size markers, M_r in thousands, are depicted on the left. Closed and open arrows indicate, respectively, the positions of the $\sim 90\text{K}$ polypeptide and the deglycosylated species ($\sim 50\text{K}$).

teins in addition to the 90K species (not shown). Several other faintly reactive bands (approximately 80K, 45K and 23K) were not competed with peptide, and were also seen in blots performed with preimmune rabbit sera or with only the anti-rabbit IgG alkaline phosphatase conjugate (not shown).

Subsequent to generation of antisera to the synthetic peptide, rabbit antibody to a β -galactosidase–CGD fusion protein (construct 3 in Fig. 2) was raised. This antiserum, which manifested less cross-reactivity to other proteins, detected the same 90K species in neutrophils (Fig. 3*b*). Detection is not competed by excess peptide (not shown); this presumably indicates that the antifusion protein antisera recognizes epitopes distinct from those detected by the anti-peptide sera. Thus, independently derived antisera detect a 90K species in normal neutrophils that is absent in X-CGD.

The precise defect in the phagocyte NADPH-oxidase system in X-CGD has not been defined. A consistent finding has been the absent spectrum of the neutrophil *b* cytochrome^{8,9}, thought to be a terminal component of the electron transport chain of the oxidase¹. Concurrent with the development of antisera to the X-CGD protein, neutrophil cytochrome *b* was isolated by a new procedure by Parkos *et al.*⁴. In this study, cytochrome *b* solubilized from neutrophil plasma membranes purified as a complex of heavily glycosylated 90K and nonglycosylated 22K species, rather than as a single component of 11–120,000K as variously reported^{10–14}. Upon deglycosylation the 90K species has an apparent relative molecular mass of approximately 50K (ref. 4). After submission of this manuscript, Segal independently reported the existence of 90K and 22K components in preparations of purified *b* cytochrome²². Figure 4 shows a Western blot analysis of purified cytochrome *b* using antisera to the synthetic peptide (panel *a*) and the β -galactosidase–CGD fusion protein (panel *b*). Antisera reacted with the 90K protein and its deglycosylated 50K form and did not react with the 22K species. The 90K species present in solubilized neutrophils corresponds in size and appearance to that contained in purified cytochrome *b* (Fig. 4*a*). Reactivity of high molecular mass species in the cytochrome preparations, particularly after deglycosylation, probably reflects aggregation of the purified protein.

Our findings provide immunological and biochemical evidence for the contention that our previously reported X-CGD mRNA sequence represents the authentic product of the relevant gene, and firm support for the functional role of the neutrophil cytochrome *b* in the respiratory burst of phagocytes, as initially proposed by Segal and co-workers⁸. The assignment of a ~90K neutrophil membrane glycoprotein as the X-CGD gene product and a component of the cytochrome *b* complex is entirely consistent with features of the cDNA sequence and resolves several aspects of the relationship of the predicted gene product and cytochrome *b* spectrum. Specifically, the inferred signal peptide following the position 208–210 AUG, the presence of at least one large hydrophobic segment (amino acids 83–110), and five potential *N*-glycosylation sites in the cDNA sequence³ are in accord with identification of a heavily modified membrane protein. The apparent discrepancy²² between the predicted X-CGD protein and the amino-acid composition of cytochrome *b* reported by Harper *et al.*¹⁴ can be readily explained by impurities acknowledged in the earlier cytochrome *b* preparations and by the presence of the 22K component in those samples subjected to amino-acid analysis. Although the determined¹⁴ and predicted compositions differ at selected amino acids, they are not vastly dissimilar. Direct amino-acid sequence or composition analysis of the 90K component of cytochrome *b*, free of contamination with 22K polypeptide, would be useful in the final resolution of these issues.

The X-CGD protein shows no homology to known proteins, including other cytochromes, notwithstanding a short stretch of uncertain significance that resembles a cytochrome P-450 (ref. 15). Our data predict that the 22K polypeptide of the purified cytochrome *b* preparations^{4,22} may contain the amino-acid residues necessary for retaining the protoporphyrin IX and, hence, characteristic cytochrome spectrum and electron transfer function. Furthermore, analysis of the hydrodynamic and cross-linking properties of the purified cytochrome *b* by two of us (C.P. and A.J.) suggests that it may be heterodimeric. Since the cytochrome *b* spectrum is also absent in an X-CGD patient with an interstitial deletion that removes the sequence encoding the C-terminal 41 amino acids of the 90K protein (ref. 3 and S.O., unpublished), the C-terminal domain of the X-CGD protein may bear the site of the putative subunit interaction. In this model interaction is critical for maintenance of the characteristic haem spectrum and for stability of the 22K component within the phagocytic cell, and also accounts for the consistent absence of the cytochrome *b* spectrum and 22K protein in classical X-CGD^{4,22}. The absence of both cytochrome *b* components in X-CGD, therefore, results from genetic deficiency of only one protein, that encoded by the gene which resides at Xp21.

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Note added in proof: Protein sequencing by Teahan *et al.*²³, while providing confirmation for the identity of the cDNA, demonstrates that the region upstream of the 208–210 AUG must be translated *in vitro*. Examination of genomic sequences in this region has revealed two errors in our published cDNA sequence: a C-insertion possibly due to a reverse transcription mistake, such that an AUG codon is present immediately before the N-terminal glycine of the determined protein; and a GTC → GCT (Val → Ala) at amino-acid residue 13. These data (finally) assign that true N terminus of the protein and indicate that a cleavable signal peptide does not exist contrary to the above speculations and those of Creighton⁶.

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4. Parkos, C. A., Allen, R. A., Cochrane, C. G. & Jesaitis, A. J. *J. clin. Invest.* (in the press).
5. Kozak, M. *Cell* **47**, 481–483 (1986).
6. Creighton, T. E. *Nature* **324**, 21 (1986).
7. Kreig, P. A. & Melton, D. A. *Nucleic Acids Res.* **12**, 7057–7070 (1984).
8. Segal, A. W. *et al. New Engl. J. Med.* **308**, 245–251 (1983).
9. Bohler, M. *et al. J. clin. Immun.* **6**, 136–145 (1986).
10. Harper, A. M., Dunne, M. J. & Segal, A. W. *Biochem. J.* **219**, 519–527 (1984).
11. Pember, S. O., Heyl, B. L., Kinkade, J. M. Jr & Lambeth, J. P. *J. biol. Chem.* **259**, 10590–10595 (1984).
12. Serra, M. C. *et al. Biochim. biophys. Acta* **788**, 138–146 (1984).
13. Lutter, R. *et al. J. biol. Chem.* **260**, 2237–2244 (1985).
14. Harper, A. M., Chaplin, M. F. & Segal, A. W. *Biochem. J.* **227**, 783–788 (1985).
15. Ashworth, A., Shephard, E. A. & Phillips, I. R. *Nature* **322**, 599 (1986).
16. Young, R. A. & Davis, R. W. *Proc. natn. Acad. Sci. U.S.A.* **80**, 1194–1198 (1983).
17. Ruther, U. & Muller-Hill, N. *Embo J.* **2**, 1791–1794 (1983).
18. Laemmli, U. K. *Nature* **227**, 680–685 (1970).
19. Towbin, H., Staehlin, T. & Gordon, J. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4350–4354 (1979).
20. Boyum, A. *Scand. J. clin. Lab. Invest.* **21** (Suppl. 97), 77–89 (1968).
21. Bradford, M. *Analyt. Biochem.* **72**, 248–254 (1976).
22. Segal, A. W. *Nature* **326**, 88–91 (1987).
23. Teahan, C., Rowe, P., Parker, P., Totty, N. & Segal, A. W. *Nature* **327**, 720–721 (1987).

The X-linked chronic granulomatous disease gene codes for the β -chain of cytochrome *b*₂₄₅

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Chronic granulomatous disease (CGD) is a rare inherited disorder associated with a profound predisposition to infection due to the lack of a microbicidal oxidase system in the phagocytes of these patients¹. This syndrome is most commonly inherited through a defect on the X chromosome and the only clearly defined component of the oxidase system, the very unusual cytochrome *b* (*b*₂₄₅), has been shown to be missing from the cells of these patients^{2,3}. This cytochrome is a heterodimer composed of an α -chain of relative molecular mass (*M_r*) 23,000 (23K) and a 76–92K β -chain; neither are detectable in neutrophils from X-linked CGD subjects. The defective X-CGD gene has recently been cloned⁴ by 'reverse genetics'⁵ but the protein predicted from the proposed complementary DNA sequence was not identified. We have purified the β -chain of the cytochrome and sequenced 43 amino acids from the N terminus. Almost complete homology was obtained between this sequence and that of the complementary nucleotides 19–147 of the sequence of the X-CGD gene, originally designated as a non-coding region.

The identification of the gene responsible for X-linked CGD⁴ has aroused considerable interest in the identity of the protein for which it codes. It was predicted to consist of 468–486 amino acids, with the initiation codon at base number 322, and considered unlikely to be related to cytochrome *b*₂₄₅. Having successfully separated the two subunits of the cytochrome, the sequence of the 43 N-terminal amino acids was determined (Fig. 1). A sequence of bases that would encode these 43 amino acids was identified in the X-CGD gene (Fig. 2) except for residue 13 at which Ala replaces Val.

Royer-Pokora *et al.*⁴ concluded that translation of the X-CGD mRNA probably started at the second in-frame AUG codon at position 322, rather than that at position 208, because it more closely resembled the consensus sequence for functional initiator codons defined by Kozak. Creighton has suggested that translation might start at the first AUG and that the 38 extra amino acids contained a signal sequence for targeting of the protein to its eventual subcellular location⁶. Doubts were raised by

1. Tauber, A. I., Bornegaard, N., Simons, E. & Wright, J. *Medicine* **62**, 286–369 (1983).
2. Baehner, R. L. *et al. Proc. natn. Acad. Sci. U.S.A.* **83**, 3398–3401 (1986).
3. Royer-Pokora, *et al. Nature* **322**, 32–38 (1986).

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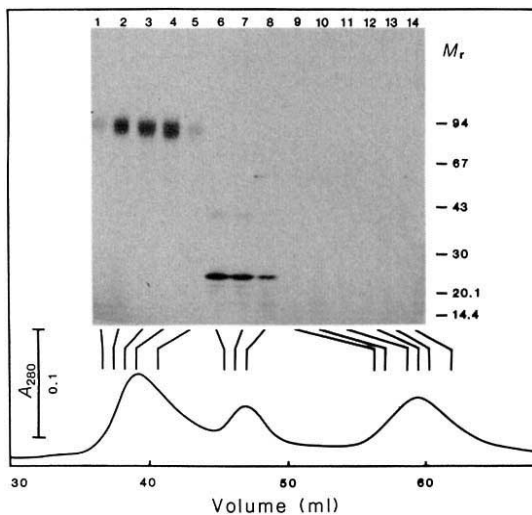


Fig. 1 Separation of the two subunits of cytochrome b_{-245} by gel filtration chromatography. The cytochrome was extracted from granulocytes and chromatographed as described previously. It was further purified by centrifugation into a linear sucrose gradient (10–50% w/v, 35 ml, containing 50 mM Tris/HCl pH 6.7 and 0.1% Triton N101 (Sigma)) at 130,000g for 20 h in a Sorvall TV-850 vertical rotor. The light cytochrome band separated from a denser precipitate of colourless protein. It was aspirated from above, diluted 1 in 5 with H_2O , bound to a 1 ml heparin agarose column, eluted with 1 ml 5% SDS in H_2O and incubated at 37°C for 18 h. An aliquot of 15 nmol was chromatographed at 18 ml h^{-1} on a 1.6 × 50 cm Superose 12 FPLC column equilibrated with 20 mM sodium phosphate pH 7.4 containing 1% SDS. The elution profile (below) revealed three peaks of absorption. Fractions indicated were subjected to SDS/PAGE on a 12% gel and silver stained. The 76–92K β -chain and the 23K α -chain subunits were clearly separated, eluting in the first and second peaks respectively. Protein was not demonstrated in the third peak.

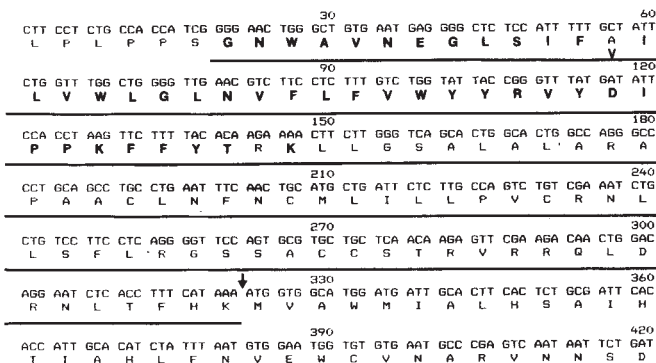


Fig. 2 N-terminal amino-acid sequence of the β -subunit of cytochrome b_{-245} compared with the 5' region of the X-CGD cDNA sequence. The β -subunit (600 pmol from pooled fractions run in tracks 2–4 in Fig. 1) was sequenced in an Applied Biosystems 477A Liquid Pulse Sequencer with on-line PTH analysis. The observed sequence (with a starting signal of 160 pmol) is shown in bold type. The sequence, both observed and derived, that is additional to the derived sequence previously published⁴, is underlined.

Cavener regarding the accuracy of both these putative start codons because of their unusual flanking nucleotides⁷.

Eukaryotic proteins commence with a methionine, the codon for which (AUG) is not observed 5' to the sequence for the mature protein. This makes it highly unlikely that the sequence of the complete gene has been obtained, almost certainly as a result of a strong stop for reverse transcriptase, a possibility initially commented upon by Royer-Pokora *et al.* The amino

acids coded for 5' to the end of the mature protein probably represent the tail of the signal peptide⁸ required to direct this molecule to its location in the plasma membrane and the membrane of the specific granules⁹.

The predicted protein has a molecular mass of 65K and the 101 amino acids 5' to the previously described sequence are predominantly hydrophobic. The calculated *pI* of the protein sequence is 9.7. No obvious clues are provided by the structure as to the function of this subunit.

This study identifies the product of the X-CGD gene as the β -chain of cytochrome b_{-245} and defines the genetic basis for the observed absence of this molecule in X-linked CGD. Standard techniques of protein purification and sequencing were required to supplement 'reverse genetics'⁵, to determine the complete amino-acid sequence of the mature protein and to identify the incomplete message at the 5' end of the gene.

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1. Quic, P. G., White, J. G., Holmes, B. & Good, R. A. *J. clin. Invest.* **46**, 668–679 (1967).
2. Segal, A. W. *et al. New Engl. J. Med.* **308**, 245–251 (1983).
3. Segal, A. W. *Nature* **326**, 88–91 (1987).
4. Royer-Pokora *et al. Nature* **322**, 32–38 (1986).
5. Orkin, S. H. *Cell* **47**, 845–850 (1986).
6. Creighton, T. E. *Nature* **324**, 21 (1986).
7. Cavener, D. R. *Nature* **325**, 21 (1987).
8. von Heijne, G. *J. molec. Biol.* **184**, 99–105 (1985).
9. Garcia, R. C. & Segal, A. W. *Biochem. J.* **219**, 233–242 (1984).

Loss of alleles of loci on the short arm of chromosome 3 in renal cell carcinoma

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Loss of genes at specific chromosomal loci is a characteristic of retinoblastoma¹, Wilms' tumour¹¹, transitional cell carcinoma of the bladder², embryonal tumours³ and small cell carcinoma of the lung^{4–7}. The significance of nonrandom gene loss in these neoplasms is that gene loss on one chromosome may uncover null mutations at corresponding loci of the homologous chromosome^{8,9}. Loss of specific gene products from somatic cells may be critical in the origin or evolution of certain human tumours. Clues to identification of new loci of gene loss in common adult solid tumours may be found in literature that describes chromosomal abnormalities in rare heritable cancers. Karyotypes of tumours in two families with hereditary renal carcinoma showed translocations involving the short arm of chromosome 3 (refs 10 and 11). We have examined tumours from 18 patients with non-hereditary renal cell carcinomas and found loss of alleles at loci on the short arm of chromosome 3 in all eleven of the patients who could be evaluated.

Our method for detecting allele loss in renal cell carcinoma was based on methods developed for study of the genetics of retinoblastoma¹. Inherited differences in nucleotide sequence on homologous chromosomes can be detected in human DNA that has been digested with appropriate restriction endonucleases, separated by agarose gel electrophoresis, transferred to filter membranes and hybridized with recombinant DNA probes¹². Each recombinant DNA probe defines a locus; these probes are used to compare loci on homologous chromosomes

Table 1 Alleles in normal kidney and renal cell carcinoma tissues

Patient no.	Tissue	Age	Histology	Stage	Alleles present					
					3p	3p	3p	3q	11p	15
					<i>DNF15S2</i>	<i>D3S2</i>	<i>D3S3</i>	<i>D3S1</i>	<i>HRAS1</i>	<i>D15S1</i>
1	N	52	Clear cell	IV	1/2	1/2	—	—	1/2	—
	T				1/	1/2	—	—	1/2	—
2	N	63	Clear cell	IV	—	—	—	—	—	—
	T				—	—	—	—	—	—
3	N	50	Clear cell	I	—	—	—	1/2	—	1/2
	T				—	—	—	1/2	—	1/2
4	N	63	Clear cell	IV	—	—	—	—	—	1/2
	T				—	—	—	—	—	1/2
5	N	59	Clear cell	I	1/2	—	1/2	—	—	1/2
	T			/2	—	/2	—	—	—	1/2
6	N	62	Granular cell	IV	—	—	—	1/2	1/2	1/2
	T				—	—	—	1/2	1/2	1/2
7	N	64	Clear cell	IV	1/2	1/2	—	—	1/2	1/2
	T				1/	/2	—	—	1/2	1/2
8	N	54	Clear cell	IV	1/2	1/2	—	1/2	—	—
	T				1/	/2	—	1/2	—	—
9	N	67	Clear cell	IV	1/2	—	1/2	1/2	—	1/2
	T				1/	—	1/2	1/1/2	—	1/2
10	N	48	Granular cell	IV	—	—	—	—	1/2	—
	T				—	—	—	—	1/2	—
11	N	56	Clear cell	IV	1/2	—	—	—	—	1/2
	T				1/	—	—	—	—	1/2
12	N	60	Clear cell	IV	—	—	—	—	1/2	—
	T				—	—	—	—	1/2	—
13	N	46	Clear cell	IV	1/2	1/2	—	—	1/2	1/2
	T				1/	/2	—	—	1/2	1/2
14	N	57	Clear cell	IV	—	1/2	—	—	—	—
	T				—	1/	—	—	—	—
15	N	44	Granular cell	IV	1/2	1/2	—	—	1/2	—
	T				/2	1/	—	—	/2	—
16	N	30	Clear cell	IV	—	1/2	—	1/2	—	—
	T				—	1/1	—	1/2	—	—
17	N	52	Clear cell	IV	—	—	—	—	—	1/2
	T				—	—	—	—	—	1/2
18	N	50	Clear cell	IV	1/2	1/2	—	—	—	1/2
	T				1/	1/	—	—	—	1/2

The presence of alleles was determined by Southern hybridization to 3p gene probes as in Fig. 1 legend, and by densitometric analysis, as in Fig. 2 legend. Alleles at each locus are designated 1 or 2 according to decreasing length. For example alleles 1 and 2 (1/2 above) of *DNF15S2* were present in the normal kidney (N) of patient 1; allele 1 (1/) of *DNF15S2* was present in the renal tumour (T) of patient 1, whereas allele 2 of *DNF15S2* was lost from the renal tumour of this patient. Blots were also hybridized with the following probes: pUCEJ (*HRAS1*) (refs 27 and 28), pMS1-14 (*D15S1*) (refs 24 and 29) and phage HS3 (*D3S1*) (refs 23 and 30). An entry '—' indicates that the normal and tumour DNA was tested and found not to be heterozygous. Stage was determined as in ref. 14.

in normal and neoplastic tissues of individual cancer patients. This method can be used to detect gene loss in neoplasms that cannot be detected by karyotype analysis.

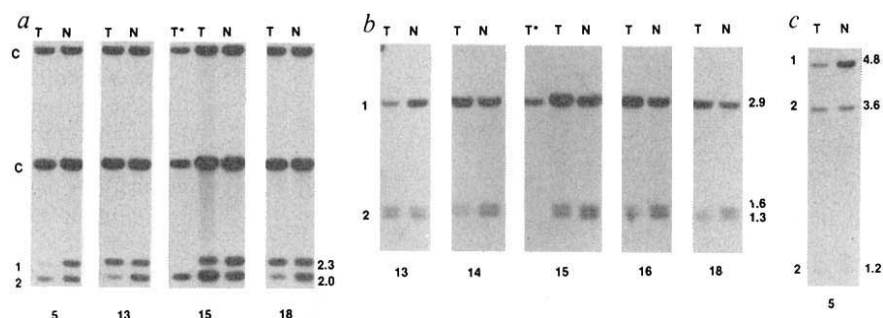
Renal cell carcinoma is the most common cancer of the kidney¹³ and accounts for 3% of adult malignancies¹⁴, but its cause is unknown. Analyses of karyotypes of tumours from patients with sporadic renal cell carcinoma have shown frequent clonal rearrangements of the short arm of chromosome 3 with the breakpoint clustering between 3p11 and 3p21 (refs 15–18). The precise nature of the change in 3p in renal cell carcinoma has not been identified. Because of the difficulties inherent in karyotypic analysis of solid tumours¹⁹ and the increased resolution afforded by analysis of restriction fragment length polymorphisms (RFLPs), particularly the ability to detect somatic gene loss, we compared restriction fragment length polymorphisms on the short arm of chromosome 3 in neoplastic and somatic tissues of patients with sporadic renal cell carcinoma.

We isolated high-molecular-weight DNA from 18 primary renal cell carcinomas and corresponding normal kidneys, digested these samples with restriction enzymes, separated the resultant fragments by electrophoresis through agarose gels and transferred the DNA fragments to nylon membranes. Each of

these paired DNA samples was then independently hybridized to three recombinant DNA probes which have been mapped to the short arm of chromosome 3 and which reveal RFLPs. The probe pH3H2 (*DNF15S2*) (refs 20–23) detects a polymorphic locus at 3p21. The probe p12-32 (*D3S2*) (refs 23 and 24) detects a polymorphic locus on 3p. The probe pMS1-37 (*D3S3*) (refs 23–25) detects a polymorphic locus at 3p14.

Representative results of the constitutional and tumour genotypes of six of the patients are shown in Fig. 1. Results of tests with the probe for *DNF15S2* are in Fig. 1a. In four patients heterozygous for this RFLP there was significant reduction in signal intensity in tumour tissue of either allele 1 or 2. Patients 5 and 15 showed loss of allele 1; patients 13 and 18 showed loss of allele 2. Fig. 1b shows the results of tests with the probe for *D3S2*. In five patients heterozygous for this restriction fragment length polymorphism there was significant reduction in signal intensity in one allele of the tumour compared to the control renal tissue. In patient 13 there was significant reduction in signal intensity of allele 1. In patients 14, 15, 16 and 18 there was significant reduction in signal intensity of allele 2 in tumour tissue. Results of tests with the probe for *D3S3* are in Fig. 1c. Patient 5 showed loss of allele 1.

Fig. 1 Southern hybridization of 3p probes to normal kidney (N) and renal cell carcinoma (T) DNA. T* indicates a renal tumour (first passage) grown in an immunodeficient mouse. Patient numbers are shown below the blots, and sizes of the polymorphic bands in kilobases (kb) are shown on the right. Alleles at each locus are designated 1 or 2 according to decreasing length; C indicates a constant invariant band. Selected blots, heterozygous for the polymorphism in normal DNA, are shown. *a*, *Hind*III digest, probe for *DNF15S2*; *b*, *Msp*I digest, probe for *D3S2*; *c*, *Msp*I digest, probe for *D3S3*.



Methods. High-molecular-weight DNA was prepared from minced renal cell carcinomas and normal renal tissues of 18 patients³¹. Before nephrectomy, patient 2 had been treated with interferon and medroxyprogesterone acetate, patient 9 had been treated with interleukin-2 plus lymphokine-activated killer cells, patient 10 had been treated with medroxyprogesterone acetate, patient 11 had been treated with interferon and vinblastine and patient 14 had been treated with megestrol acetate. Other patients had not been previously treated with chemotherapeutic drugs or adoptive immunotherapy. The specimens were pulverized in a tissue grinder, digested with proteinase K at 37 °C for 4 h and extracted with phenol and chloroform as described elsewhere³¹. DNA (7 µg) was digested with restriction enzymes and the resulting fragments were separated by electrophoresis through agarose gels and then transferred to nylon filters. The following gene fragment probes were used: a 1.0-kb *Sal*I-*Hind*III fragment derived from the recombinant plasmid p12-32 (*D3S2*) containing a random human genomic sequence; a 2.0-kb *Hind*III fragment derived from the recombinant plasmid pH3H2 (*DNF15S2*) containing a random human genomic sequence; a 2.5-kb *Hind*III-*Eco*RI fragment derived from the recombinant plasmid pMS1-37 (*D3S3*) containing a random human genomic sequence. The probe fragments were separated by electrophoresis and 'oligolabelled' directly in gel to a specific activity of $>10^9$ d.p.m. µg⁻¹ as described^{32,33}. Details of hybridization, post-hybridization washing and autoradiography are available on request.

To quantitate the loss of 3p alleles in tumour tissue we measured the hybridization signals shown in Fig. 1 by scanning autoradiographs with a densitometer (Fig. 2) and thus determining the ratios of hybridization of restriction fragments obtained from the normal and tumour DNA. The ratios were used to obtain a factor to correct for differences in DNA loading. Densitometric analysis confirmed the reduction in signal intensity of one allele in each of the patients analysed in Fig. 1. With the probe for *DNF15S2* the results were: in patient 13, allele 2 was decreased by 54%; in patient 15, allele 1 was decreased by 32%; and in patient 18, allele 2 was decreased by 46%. With the probe for *D3S2* the results were: in patient 14, allele 2 was decreased by 60% whereas in patient 16, allele 2 was decreased by 40%.

The hybridization signal of the remaining 3p allele in patient 16 is of about double intensity in the tumour cells and is unchanged in patients 13, 14, 15 and 18. Loss of 3p alleles in tumour cells of patient 16 was associated with duplication of the retained allele, whereas simple loss of 3p alleles occurred in tumour cells of patients 13, 14, 15 and 18.

Table 1 presents all the genotypic data obtained in this study. Patients 1, 5, 7, 8, 9, 13, 15 and 18 were constitutionally heterozygous at two loci on the short arm of chromosome 3. Patients 11, 14 and 16 were constitutionally heterozygous at one locus on the short arm of chromosome 3. In nine cases (patients 5, 7, 8, 11, 13-16 and 18), at each locus that was heterozygous for a 3p polymorphism, one of the two codominant alleles was lost in the process of tumorigenesis; in two patients (1 and 9) one probe to 3p (probe for *DNF15S2*) detected loss of heterozygosity whereas a second probe to 3p did not detect loss. It was not possible to detect accurately loss of alleles at 3p in renal tumours of patients that were constitutionally homozygous.

The significant decrease in signal intensity from one of two alleles in 11 out of 11 evaluable (heterozygous) patients with renal cell carcinoma reflects the fact that either all renal carcinoma cells have lost 3p alleles and that the residual signal originates from stromal and inflammatory cells contaminating the tumour, or that some but not all renal cancer cells have lost 3p alleles. The results of differential cell counts on these tumours support the concept that the residual signal originates from inflammatory cells present in renal tumours. In an analysis of 30 renal tumours the mean percentage of tumour cells was $40\% \pm 3.4\%$; the remaining cells were predominantly lymphocytes²⁶. One approach to determining whether the residual signal in the tumours originates from non-malignant stromal

tissue would be to test renal tumours growing in immunodeficient mice. The proliferative advantage of the tumour cells would be expected to select for pure populations of tumour cells. A renal tumour grown in an immunodeficient mouse was available from patient 15. Figure 1a and b (patient 15) show a comparison of the DNA from the xenograft, the primary renal tumour and the normal kidney hybridized with probes for *D3S2* and *DNF15S2*. The primary renal tumour, when tested with probe for *D3S2*, showed a 29% decrease in intensity of allele 2; allele 2 is completely lost in the xenograft. When tested with a probe for *DNF15S2* the primary renal tumour showed a 32% decrease in intensity of allele 1. No allele 1 signal is present in the xenograft. This result supports the idea that in patient 15 the decreased intensity of the 3p alleles reflects signal originating from residual contaminating leukocytes and that in fact all renal carcinoma cells have lost the 3p alleles.

To test whether the loss of heterozygosity in these renal cell carcinomas was specific to chromosome 3p or part of a more general reduction to homozygosity we hybridized the filters from which these data were drawn, and other filters, to probes homologous to the long arm of chromosome 3 and also to human chromosome 11 and 15 (Table 1). Gene loss at 11p was observed in 1 out of 7 informative (heterozygous) patients with renal cell carcinoma (patient 15, Table 1). The probe to 11p had been used to demonstrate loss of genes at 11p in Wilms' tumour¹ and transitional cell carcinoma of the bladder². Therefore a probe which detects non-random gene loss in Wilms' tumour and transitional cell carcinoma of the bladder did not detect such loss in renal cell carcinoma. Tumour tissue was heterozygous in 10 out of 10 informative patients tested with a probe to chromosome 15 and 5 out of 5 informative patients tested with a probe to the long arm of chromosome 3. Probes to sequences on the long arm of chromosome 3, chromosome 11 and 15 did not show evidence for a general reduction to homozygosity.

Our results indicate that, in agreement with studies on bladder cancer², it is possible to detect gene loss in adult primary solid neoplasms by analysis of RFLPs. Gene loss in adult primary solid neoplasms can be demonstrated in neoplasms that contain appreciable quantities of normal non-malignant stromal or inflammatory cells. The analysis of such mixed cell populations is aided by simultaneous evaluation of these tumours grown in immunodeficient mice.

The frequency (100%) of loss of 3p sequences in renal cell carcinoma is greater than the frequency of loss of 11p sequences in bladder carcinoma (42%)² and of loss of 11p sequences in

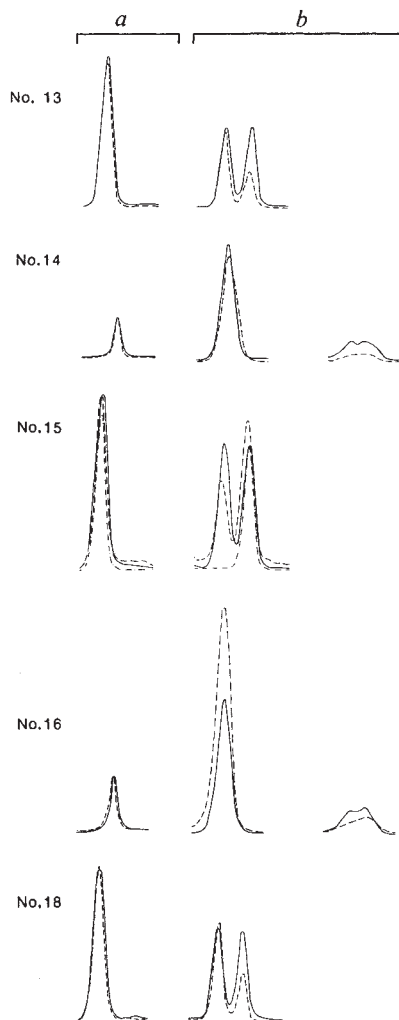


Fig. 2 Densitometric scans of Southern hybridizations with 3p probes. The tracings were adjusted for differences in amount of DNA in the lanes as described below. In each row, the left-hand panel (a) shows the control chromosome; the right-hand panel (b) shows chromosome 3p. Solid lines, normal tissue; dashed lines, tumour tissue; dashed and dotted line, tumour tissue from an immunodeficient mouse. Patient number is to the left of the tracing. Patients 13, 15 and 18 were tested with the probe for *DNF15S2*. Panel a shows the signal from the 3.8 kb constant band from chromosome 1; panel b shows the 2.3- and 2.0-kb bands from chromosome 3p. Patients 14 and 16 were tested with probes for *D3S2* and for *D15S1*. Panel a shows the signal from the 12.0-kb band from chromosome 15; panel b shows the 2.9-, 1.6- and 1.3-kb bands from chromosome 3p.

Methods. Grain density on autoradiographs was quantitated by densitometric tracing using an LKB laser Ultrascan densitometer and an Appligation II computer program (Dynamic Solutions Corp). For patients 13, 15 and 18 band density was measured on the blots shown in Fig. 1a. The data was analysed to determine the ratio of the hybridization signals from constant bands from chromosome 1 in normal tissue and tumour DNA. This factor was used to correct for differences in amount of DNA in the lanes. The tracings show the signal from chromosome 1 and chromosome 3 corrected for these differences. For patients 14 and 16 band density was measured on the blots shown in Fig. 1b. Then the blots were freed of probe and rehybridized with a probe for *D15S1*. The blots were scanned to determine the ratio of the hybridization signal from normal tissue and tumour DNA. This factor was used to correct for differences in amount of DNA in the lanes.

Wilms' tumour (55%)¹⁹. Loss of 3p sequences in the tumours of all informative (heterozygous) patients with renal cell carcinoma indicates that loss of heterozygosity in the 3p region is a non-random alteration in this carcinoma and therefore it may be important in the origin or evolution of this tumour. Loss of alleles on the short arm of chromosome 3 is not confined to renal cell carcinoma. Karyotype analysis has shown a non-random deletion at 3p in small-cell lung carcinoma^{4,5,7}. Indeed one of the recombinant DNA probes to 3p (probe for *D3S3*) used in this study to demonstrate allele loss on 3p in renal cell carcinoma has been used by others to demonstrate allele loss in small-cell lung carcinoma⁶; these observations in small-cell lung carcinoma have been confirmed in this laboratory (H.B., B. Johnson, A. Gazdar, J. Minna and B.Z., unpublished observations). These data, demonstrating allele loss in both renal cell carcinoma and small cell lung carcinoma raise the question of whether a recessive oncogene located on 3p is involved in the origin or evolution of both renal cell carcinoma and small-cell lung carcinoma. This suggestion, that a recessive oncogene might be involved in tumorigenesis for several histologically different neoplasms, has previously been made on the basis of the observation of loss of genes at 11p in Wilms' tumour and embryonal tumours³.

The loss of alleles at loci on the short arm of chromosome 3 observed in renal cell carcinoma, by analogy to retinoblastoma^{1,8,9} may indicate that molecular analysis of the 3p region should ultimately disclose a null mutation at one 3p allele associated with loss of the wild-type allele. The function of a 'renal carcinoma gene' might be to enable the renal cell to respond appropriately to environmental stimuli. Future studies should develop additional molecular probes for 3p and begin attempts to clone genes in this region. Such genes may be essential for normal differentiation of renal cells and the precursors of small-cell lung carcinoma cells.

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- Strong, L. C. in *Genetics in Clinical Oncology* (eds Chaganti, R. S. K. & German, J.) 39-59 (Oxford University Press, Oxford, 1985).
- Fearon, E. R., Feinberg, A. P., Hamilton, S. H. & Vogelstein, B. *Nature* **318**, 377-380 (1985).
- Koufos, A. et al. *Nature* **316**, 330-335 (1985).
- Whang-Peng, J. et al. *Cancer Genet. Cytogenet.* **6**, 119-134 (1982).
- Whang-Peng, J. et al. *Science* **215**, 181-183 (1981).
- Naylor, S. L., Minna, J., Johnsons, B. & Sakaguchi, A. Y. *Am. J. Hum. Genet.* **36**, 355 (1984).
- De Leij, L. et al. *Cancer Res.* **45**, 6024-6033 (1985).
- Friend, S. H. et al. *Nature* **323**, 643-650 (1986).
- Cavenee, W. K. et al. *Science* **228**, 501-503 (1985).
- Cohen, A. J. et al. *New Engl. J. Med.* **301**, 592-595 (1979).
- Pathak, S., Strong, L. C., Ferrell, R. E. & Trindade, A. *Science* **217**, 939-941 (1982).
- Botstein, D., White, R., Skolnick, M. & Davies, R. W. *Am. J. Hum. Genet.* **32**, 314-331 (1980).
- Paulson, D. F., Perez, C. A. & Anderson, T. in *Cancer: Principles and Practice of Oncology* 2nd edn (eds DeVita, V. T. Jr, Hellman, S. & Rosenberg, S. A.) (Lippincott, Philadelphia, 1985).
- De Kernion, J. B. in *Campbell's Urology* 5th edn (eds Walsh, P. C., Gittes, R. F., Perlmutter, A. D. & Stamey, T. A.) 1294-1342 (Saunders, Philadelphia 1986).
- Wang, N. et al. *Am. J. hum. Genet.* **35**, 73A (1983).
- Yoshida, M. A. et al. *Cancer Genet. Cytogenet.* **19**, 351-354 (1986).
- Yoshida, M. A. et al. *Cancer Res.* **46**, 2139-2147 (1986).
- Teyssier, J. R. et al. *J. natn. Cancer Inst.* **77**, 1187-1195 (1986).
- Solomon, E. *Nature* **309**, 111-112 (1984).
- Harper, M. E. & Saunders, G. F. *Chromosome* **83**, 431-439 (1981).
- Carritt, B., Welch, H. M. & Parry-Jones, N. J. *Am. J. hum. Genet.* **38**, 428-436 (1986).
- Donlon, T. A. & Magenis, R. E. *Cytogenet. Cell Genet.* **37**, 454-455 (1984).
- Kidd, K. K. & Gusella, J. *Cytogenet. Cell Genet.* **40**, 107-127 (1985).
- Barker, D., Schafer, M. & White, R. *Cell* **36**, 131-138 (1984).
- Gerber, M. J., Miller, Y. E., Drabkin, H. A. & Scoggins, C. H. *Cytogenet. Cell Genet.* **42**, 72-74 (1986).
- Belldgrun, A., Linehan, W. M., Robertson, C. N. & Rosenberg, S. A. *Surg. Forum* **37**, 671-673 (1986).
- Shih, C. & Weinberg, R. A. *Cell* **29**, 161-169 (1982).
- Goldfarb, M. P., Shimizu, K., Perucho, M. & Wigler, M. H. *Nature* **296**, 404-409 (1982).
- De Martinville, B., Schafer, M., White, R. & Francke, U. *Molec. Biol. Med.* **1**, 415-424 (1983).
- Zabel, B. U. et al. *Am. J. hum. Genet.* **34**, 153A (1982).
- Tanio, Y. et al. *J. natn. Cancer Inst.* **77**, 767-775 (1986).
- Feinberg, A. P. & Vogelstein, B. *Analyt. Biochem.* **132**, 6-13 (1983).
- Feinberg, A. P. & Vogelstein, B. A. *Analyt. Biochem.* **137**, 266-267 (1984).

Age related reactivation of an X-linked gene

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We have investigated age-related reactivation of the X chromosome by devising a model in which reactivation of a single gene in one cell among many can be identified. We have used mice with an X-autosomal translocation giving consistent non-random inactivation of the normal X (as judged by biochemical and cytogenetic techniques), that also carry a defective form of a histochemically demonstrable X-linked enzyme. When the gene for the normal enzyme was located on the inactivated normal X a uniformly negative histochemical picture would be predicted in doubly heterozygous animals. A very small proportion of enzyme-positive cells was found in young animals. This proportion increased very significantly with age, but the patch size did not change, showing that the result was not due to preferential division of enzyme-positive cells, but was instead due to the conversion of previously enzyme-negative to enzyme-positive cells. These observations provide the first evidence with a true X-linked gene for an age-related decrease in the stability of the X-inactivation mechanism.

The cellular ageing process has been attributed to the accumulation of errors in cell control mechanisms with time, although the level at which significant errors occur and the type of error involved are subjects of speculation^{1,2}. The possibility that ageing is associated with epigenetic errors leading to the reactivation of previously inactivated genes is clearly important, but is one that is difficult to demonstrate in normal animals. We have previously used X-linked enzyme histochemistry to show cellular mosaicism³, to study the clonal origin of tumours⁴ and to estimate the primordial pool size of the liver⁵. Cattanaach⁶ showed that animals which are heterozygous for an X-linked marker and also show non-random X-chromosome inactivation provide material for detection of reactivation of X-chromosome genetic material. However, Cattanaach's data related to autosomal genes inserted into the X and these seemed to behave differently from naturally X-linked genes. We reasoned that histochemistry could be used to detect reactivation of an X-linked gene in an extremely small proportion of a cell population if the reactivation led to the production of a histochemically detectable enzyme against an otherwise negative background. We have therefore applied X-linked enzyme histochemistry to study age-related reactivation

of an X-linked enzyme on a normal X chromosome. In mice with the sparse fur trait (*spf*/Y or *spf*/*spf*) a mutation of the structural gene on the X chromosome coding for the urea cycle enzyme ornithine carbamoyl transferase (OCT) leads to the production of an abnormal form of OCT⁷. This abnormal enzyme gives a negative histochemical result at pH 7, so that female mice heterozygous for *spf* show a mosaic staining in the liver with a defined OCT histochemical technique³. Cytogenetic and biochemical studies in mice with the X-autosomal translocation T(X;16)16H (Searle's translocation) have revealed no activity of the normal X chromosome beyond early embryonic stages^{8,9}. We have, therefore, studied mice doubly heterozygous for Searle's translocation and *spf*, in which the normal OCT gene was on the normal chromosome.

If there were consistent non-random inactivation of the normal X chromosome as predicted, only the abnormal OCT gene would be active in the heterozygous females and a uniformly negative histochemical picture in the liver would result. Any cell in which the normal X chromosome, or that part containing the OCT locus, were active would then stand out as an isolated positive and any change in the number of positive cells with age could be quantified.

Six young (8–10 weeks), seven adult (6–9 months) and five old (14–17 months) *spf* T16H/+ + mice were killed by ether inhalation, and blocks taken from each of the six morphologically distinct liver lobes. Four 5- μ m sections were taken at different levels of each block and stained for the presence of normal OCT. Control liver sections from chromosomally normal *spf* +/+ +, +/+ + + and *spf* +/Y mice were processed at the same time, and routinely showed the expected result of mosaicism, that is, uniform positivity and uniform negativity, respectively.

Small numbers of darkly staining OCT positive cells were identified in liver sections in each of the doubly heterozygous T16 *spf*/+ + mice studied; in most cases the positivity occurred as isolated cells, although a few small groups of positive cells were also seen (Figs 1 and 2). A quantitative analysis was made of the proportional area of positive cells in the sections, the mean patch area, the mean patch concentration and the number of cells per patch, from which was determined the number of one-cell patches and the average number of cells per patch. The mean area of individual positive and negative nucleated cells was measured separately in animals of different ages to determine whether any of the observed changes could be due to changes in cell size rather than cell number.

Striking changes were observed with age (Table 1), with an ~50-fold increase in the relative area of positive cells when mice under 10 weeks of age were compared with those over a year old ($P < 0.001$). Although the mean individual hepatocyte area (Table 2) increased with age ($P < 0.001$), there was no significant difference at any of the ages studied between the mean individual positive and negative cell areas ($P > 0.1$), so that the change in

Table 1 OCT-positive hepatocytes in *spf* T16H/+ + mice

	Young	Adult	Old
Positive area (%) (mean $\pm$ s.d.)	0.0558 $\pm$ 0.377	0.2861 $\pm$ 0.551	2.7690 $\pm$ 2.759
Patch area (μ m ²) (mean $\pm$ s.d.)	384.88 $\pm$ 418.20	262.90 $\pm$ 180.58	341.68 $\pm$ 299.36
No. of patches measured*	223 (1,350)	1,655 (1,580)	8,232 (1,070)
Patches per field (mean $\pm$ s.d.)	0.14 $\pm$ 0.71	1.05 $\pm$ 1.62	7.63 $\pm$ 5.61
Proportion of one-cell patches†	71.8% (265)	89.9% (1,488)	83.7% (6,892)
Average no. of cells per patch	1.42 $\pm$ 0.72	1.15 $\pm$ 0.39	1.16 $\pm$ 0.09

Numbers in parentheses show no. of fields examined (*) and no. of one-cell patches counted (†).

Summary of data on the change with age of the occurrence of OCT-positive hepatocytes in *spf* T16H/+ + mice. The number of OCT-positive patches, their areas and the unstained hepatocellular areas were measured and recorded using an IBAS II image analyser. The number of cells in each patch was counted and recorded manually at magnification $\times 125$. Ten random fields were sampled from each section and the analyses made on aggregates of the 10 fields; 135 sections were sampled for the young animals, 155 for adult animals and 104 for the old mice; missing data consisted mainly of a small number of single lobes that could not be measured due to technical difficulties, but in a few cases individual sections were unavailable for sampling.

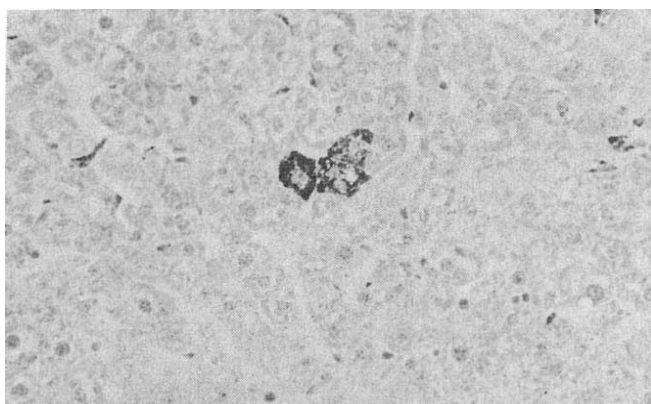


Fig. 1 High-power photomicrograph of a liver section from an adult *spf* T16H/+ female mouse stained for normal OCT showing a group of three OCT-positive cells, including one binucleate cell. Several pigment-containing Kupffer cells are also present (magnification $\times 125$).

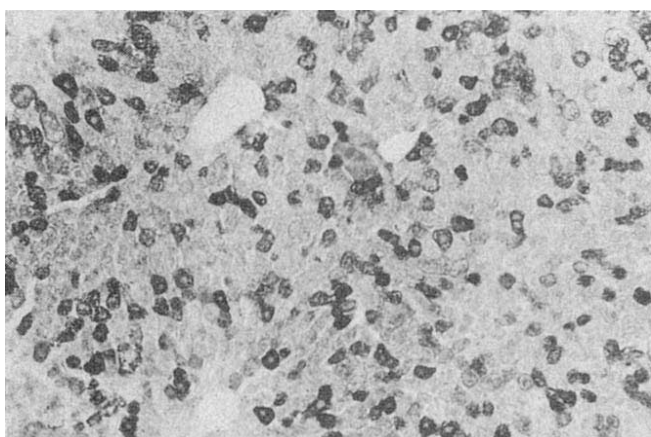


Fig. 2 Low-power photomicrograph of a liver section from an old *spf* T16H/+ female mouse stained for normal OCT showing many positive cells. Note that the cells occur predominantly as single cells although some small groups are visible ($\times 50$).

proportional area reflects change in relative cell number. During the same period (Table 1) the number of patches of positive cells increased ~ 20 -fold ($P < 0.001$), but the great majority of these were single-cell patches, quite unlike the mosaic pattern seen in heterozygous females. The observed average number of cells per patch did not change significantly with age ($0.05 < P < 0.1$). We therefore conclude that the age-related increase in the proportion of cells expressing normal OCT in mice doubly heterozygous for Searle's translocation and *spf* is not due to preferential division of enzyme-positive cells but to reactivation of the normal OCT gene in previously inactive cells or in the progeny of previously inactive cells. The rate of reactivation increases non-linearly with time.

Reactivation of X-linked genes has been much studied in cultured cells. Spontaneous reactivation is extremely rare¹⁰, but the frequency is increased following the use of 5-azacytidine, a substance that reduces the level of DNA methylation and alters gene expression^{11,12}. DNA methylation has been shown to decrease in ageing fibroblasts in culture, but to remain constant in immortal cell lines and it has been suggested that this may account for the aberrant ectopic protein production seen in these cells^{13,14}. It has recently been shown that 5-azacytidine treatment of cultured human fibroblasts reduces their lifespan, again providing support for the view that random loss of methyl groups in DNA may lead to aberrant epigenetic changes in gene expression, and that this mechanism may be an important part of the ageing process¹⁵. In addition, 5-azacytidine has been shown to reactivate the OCT gene in cultured rat hepatoma cells lines^{16,17}.

Our demonstration of age-related reactivation of an X-linked gene *in vivo* parallels Cattanach's⁶ demonstration of an age-related reactivation of autosomal genes located on the portion of chromosome 7 inserted in the X chromosome in Cattanach's translocation. Interestingly, the genes farthest from X chromosomal material were the first to be reactivated in the latter case

and the OCT gene which we have studied lies at almost the maximum possible distance from the X-chromosome inactivation centre (*Xce*)^{18,19}, which not only determines X inactivation in the early embryo, but may also be important in maintaining that inactivation during the life of the cell and its progeny. We cannot, from the study of the activity of a single gene, determine with certainty whether the reactivation observed is an isolated consequence of an individual somatic mutation, or reversal of the pattern of X inactivation, or whether it reflects progressive destabilization with age of the X-chromosome inactivation system. No examples of individual positive cells were found in any of the *spf* +/Y mice liver sections studied, so that a mutation leading to restoration of normal enzyme activity at the *spf* locus can be excluded as a possibility. The liver is known to show a low level of mitotic activity throughout adult life and to show an increase in the proportion of polyploid cells with age. We looked for evidence that cells showing restoration of enzyme activity showed a higher frequency of polyploidy than the rest of the liver, but failed to find this. Because the rate of reactivation

Table 2 Mean cell area of positive and negative cells

Age group	No. of cells measured	Mean area $\pm$ s.d. (μm^2)	
		Positive	Negative
Young	100	244.5 $\pm$ 84.1	254.5 $\pm$ 62.7
Adult	100	263.6 $\pm$ 78.8	248.6 $\pm$ 65.1
Old	100	341.6 $\pm$ 141.3	351.8 $\pm$ 143.7

Mean cross-sectional areas of positive and negative hepatocytes for each age group were estimated using an IBAS II image analyser system. The areas of 100 randomly selected nucleated cells of each type were measured for each age group. There was no significant difference between the two cell types within an age group but size increased significantly with age ($P < 0.001$). This may reflect in part the increase in the number of multinucleate cells.

appeared to increase with age and because of the close parallel between our results with a normal X chromosome and Cattanaach's⁶ with autosomal material inserted into an X chromosome, we believe that our results reflect progressive destabilization with age of the X-chromosome inactivation system.

Our study demonstrates the value of X-linked enzyme histochemistry in the investigation of age-related changes in gene action. It was necessarily carried out in animals with an X-autosomal translocation and we cannot exclude the possibility that X-chromosome instability is greater in these than in normal animals. We have, however, clearly shown that in mice with Searle's translocation there is a progressive age-related reactivation of the OCT locus on the inactivated normal X chromosome and we believe that this may reflect a general mechanism of ageing.

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- Medvedev, Zh. A. *Mech. Ageing Dev.* **14**, 1-14 (1980).
- Kirkwood, T. B. L., Holliday, R. & Rosenberger, R. F. *Int. J. Cytol.* **92**, 93-132 (1984).
- Wareham, K. A., Howell, S., Williams, D. & Williams, E. D. *Histochem. J.* **15**, 363 (1983).
- Howell, S., Wareham, K. A. & Williams, E. D. *Am. J. Path.* **121**, 426-432 (1985).
- Wareham, K. A. & Williams, E. D. *J. Embryol. exp. Morph.* **95**, 239-246 (1986).
- Cattanaach, B. M. *Genet. Res.* **23**, 291-306 (1974).
- DeMars, R., LeVan, S. L., Trend, B. L. & Russell, L. B. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1693-1697 (1976).
- Takagi, N. *Chromosoma* **81**, 439-459 (1980).
- McMahon, A. & Monk, M. *Genet. Res.* **41**, 69-83 (1983).
- Migeon, B. R., Wolf, S. F., Maren, C. & Axelman, J. *Cell* **29**, 595 (1982).
- Hors-Cayla, M. C., Heuertz, S. & Frezal, J. *Somatic Cell Genet.* **9**, 645-657 (1983).
- Jones, P. A. *Cell* **40**, 485-486 (1985).
- Wilson, V. L. & Jones, P. A. *Science* **220**, 1055-1057 (1983).
- Rosen, S. W., Weintraub, B. D. & Aaronson, S. A. *J. clin. Endocr. Metab.* **50**, 834-841 (1980).
- Holliday, R. *Exp. Cell Res.* **166**, 543-552 (1986).
- Delers, A., Szpirer, J., Szpirer, C. & Saggiomo, D. *Molec. cell. Biol.* **4**, 809-812 (1984).
- Goss, S. J. *J. Cell Sci.* **72**, 241-257 (1984).
- Johnston, P. G. & Cattanaach, B. M. *Genet. Res.* **37**, 151-160 (1981).
- Rastan, S. J. *Embryol. exp. Morph.* **78**, 1-22 (1983).

Induction of sequence-specific binding of *Drosophila* heat shock activator protein without protein synthesis

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***Drosophila* tissue culture cells stimulated by heat shock contain high levels of heat shock activator protein, which binds specifically to the heat-shock control DNA element. In contrast, nonshocked cells have low basal levels of binding activity. Here, we show that within 30 seconds of heat shock of intact cells the sequence-specific binding activity in whole cell extracts increases significantly, reaching a plateau by 5 min after the start of the shock; removal of the heat stimulus returns the activity to basal levels. Known chemical inducers of heat-shock genes elicit a similar pattern of specific binding activity. Moreover, this pattern is observed in the presence of protein synthesis inhibitors, even if the stimulus-withdrawal is repeated sequentially through five cycles. Our results are inconsistent with models which propose proteolysis as the chief means of mediating heat-shock transcriptional control. Rather, they suggest that heat shock activator pre-exists in normal cells in a nonbinding form, which is converted upon cell stimulus to a high affinity, sequence-specific binding form, most probably by a post-translational modification. This conversion may be crucial for the transcriptional activation of heat shock genes.**

The heat-shock phenomenon was discovered by Ritossa^{1,2} as an induction of new puffs in *Drosophila* salivary gland polytene

chromosomes in response to stimulation with heat or chemicals known to uncouple oxidative phosphorylation. Much progress has since been achieved in molecular studies of the heat shock genes, their transcripts and protein products³⁻⁸. In particular, deletion mapping analyses of the transcriptional regulation of heat-shock genes have identified a *cis*-acting heat shock control element (HSE), consensus sequence CT_GAA__TTC_AG (ref. 9), that is found in one or more copies 5' to each heat shock gene. In parallel, studies from our laboratory of protein-DNA interactions in nuclei¹⁰ and *in vitro*^{11,12} have identified a *trans*-acting factor (heat shock activator protein)¹¹ that binds specifically to the HSE. Heat shock activator has been purified to over 95% homogeneity (C.W., S. Wilson, T. Paisley, B. Walker, V.Z. & H. Veda, manuscript in preparation) and migrates on SDS-polyacrylamide gels with a relative molecular mass (M_r) of about 110,000 (110K). Independently, Parker and co-workers^{13,14} have isolated by *in vitro* transcription and DNA-binding assays a factor (heat shock transcription factor) which also exhibits specific binding to the HSE. Heat shock activator and heat shock transcription factor may be (or be derived from) the same protein, but important discrepancies in the M_r of these factors (initially reported to be 43K daltons¹⁵ for the latter) and in the DNA-binding ability before and after heat shock as reported here, remain to be resolved.

Previously we have shown that binding of heat shock activator to the HSE *in vivo* is tightly correlated with heat shock; the uninduced gene has a vacant HSE¹⁰. Moreover, nuclear extracts prepared from heat shocked *Drosophila* embryos and tissue-culture cells have about 20 times more HSE-binding activity than extracts of nonshocked material when assayed by binding to nuclear chromatin¹¹ or to free DNA (unpublished observations). Because of the limitation that nuclear extracts do not represent total cellular HSE-binding activity we now routinely measure this activity in whole cell NaCl extracts of normal cells and cells stimulated by heat or chemical treatment. Whole cell extracts are especially suitable for quantitative comparisons between multiple samples because variable losses inherent in subcellular fractionation are not incurred.

Figure 1a (left) shows the kinetics of induction of binding activity on the *Drosophila* hsp82-HSE region, assayed by *Escherichia coli* exonuclease III (*Exo*III) protection on a 209-base pair (bp) hsp82 promoter fragment. The assay was modified for use with whole cell extracts (see Fig. 1 legend). *Exo*III digesting from an upstream site encounters heat shock activator protein initially at position -91 (arrowhead); there is further nibbling to -82 (ref. 12), generating a doublet band characteristic of protection on the hsp82-HSE. On the other hand *Exo*III digestion of the unbound DNA fragment results in residual fragments of about half-molecule size and smaller (see ref. 12 for details of the free-DNA digestion pattern). When subsaturating amounts of heat shock activator are used in the binding assay, the fragments protected by protein and the residual fragments from unbound DNA are observed in the same gel lane. We deliberately perform our assays with excess DNA, at a DNA concentration one to two orders of magnitude above the estimated dissociation constant of 4×10^{-12} M (manuscript in preparation), so that the intensity of the specifically protected DNA fragments is a fair comparison of the relative HSE-binding activity in extracts of equivalent numbers of cells. Absolute activity measurements using crude extracts are not reliable in this and other footprinting assays due to many interfering activities.

One can observe a basal level of binding activity in normal *Drosophila* Schneider Line 2 (SL2) cells, possibly owing to recovery from the partially anoxic conditions of suspension culture in spinner flasks and to the sensitivity of the cells to experimental manipulation and centrifugation. Nevertheless, a distinct increase in specific binding reflected in increased intensity of the protected fragments mapped to -91 and -82 is

Fig. 1 *ExoIII* protection over the *hsp82*-HSE region using extracts of SL2 cells induced by *a*, heat shock; *b*, sodium salicylate; and *c*, 2,4-dinitrophenol (DNP). The duration and severity of the heat or chemical shock are indicated in the top headings.

Methods. SL2 cells were grown in spinner culture as described previously¹⁰. To apply the heat shock, 0.5 ml of mid-log-phase cells concentrated to $2.5 \times 10^7 \text{ ml}^{-1}$ were dispensed in aliquots in 1.5-ml microcentrifuge tubes and shocked in a 37.5°C bath for the times indicated before pelleting (centrifugation for 2 s). The supernatants were removed and the cell pellets frozen in liquid N_2 . Concentrated cells were similarly shocked for 15 min in baths of different temperatures before the cells were pelleted and frozen. For chemical shock, cells at $5 \times 10^6 \text{ cells ml}^{-1}$ were kept agitated in suspension after administration of sodium salicylate or DNP. At the times indicated, 4-ml aliquots were removed, concentrated to $\sim 1 \text{ ml}$, pelleted and frozen. Cells (10 ml) were also treated with the indicated doses of chemical before pelleting and freezing. Whole cell extracts were prepared from frozen pellets by thawing and repetitive pipetting in plastic tips in 5 vols of extraction solution (10 mM HEPES pH 7.9, 0.4 M NaCl, 0.1 mM EGTA, 0.5 mM DTT, 5% glycerol, 0.5 mM phenylmethylsulphonyl fluoride (PMSF)) until the suspension was visually homogeneous. The lysate was centrifuged at $100,000g$ for 5 min and the supernatant ($15 \text{ mg protein ml}^{-1}$) was used for the *ExoIII* protection assay or frozen in liquid N_2 . The time of extraction until attainment of $100,000g$ was about 10 min. No improvement of extraction of heat shock activator was detected if cells were disrupted in a Teflon and glass homogenizer or if the extraction time was increased to 40 min; also re-extraction of the $100,000g$ pellet did not yield detectable activity. For the *ExoIII* protection assay, $10 \mu\text{l}$ of extract (3×10^6 cell equivalents) were added to $40 \mu\text{l}$ of binding buffer (37.5 mM NaCl, 15 mM Tris-HCl pH 7.4, 0.1 mM EGTA, 0.5 mM dithiothreitol (DTT), 5% glycerol) and $4 \mu\text{l}$ of a mixture containing $10 \mu\text{g}$ of yeast tRNA, $10 \mu\text{g}$ of pdN₅ (Pharmacia), $1 \mu\text{g}$ fragmented *E. coli* DNA and 10 fmol (1.2 ng) of 5' ^{32}P -end-labelled DNA fragment (0.2 nM) from positions -170 to +39 of the *Drosophila hsp82* gene containing three overlapping HSE's (see Fig. 1 of ref. 12 for details). Binding was performed at 25°C for 10 min, after which samples were placed on ice, MgCl_2 was added to 2 mM, and 260 units of *ExoIII* (we note that commercial preparations vary considerably in activity) were added. *ExoIII* digestion was carried out at 30°C for 13 min. The reaction was terminated, DNA purified, electrophoresed on an 8% sequencing gel and autoradiographed as previously described¹².

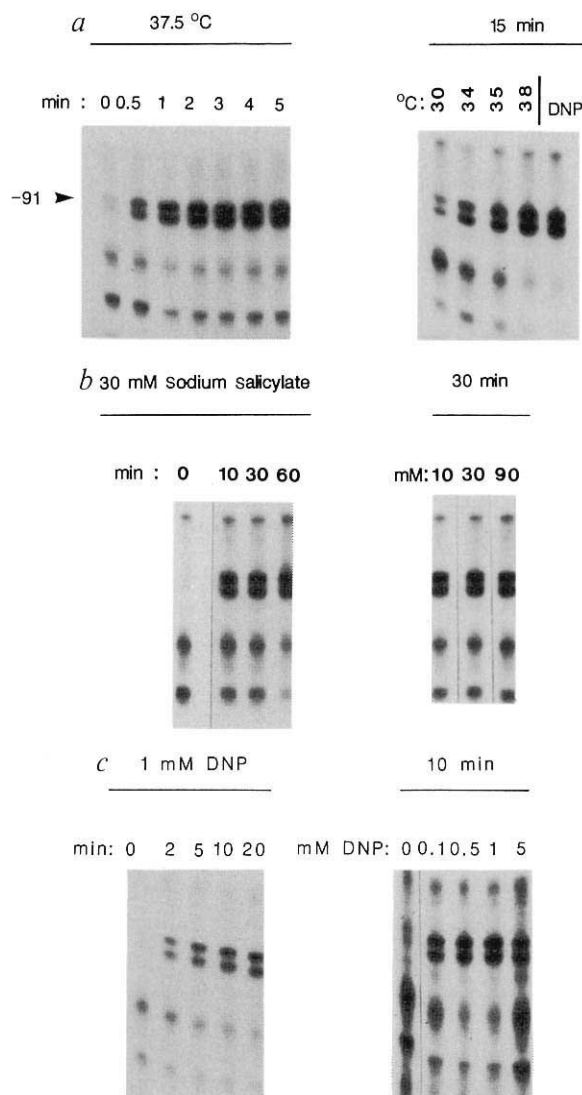
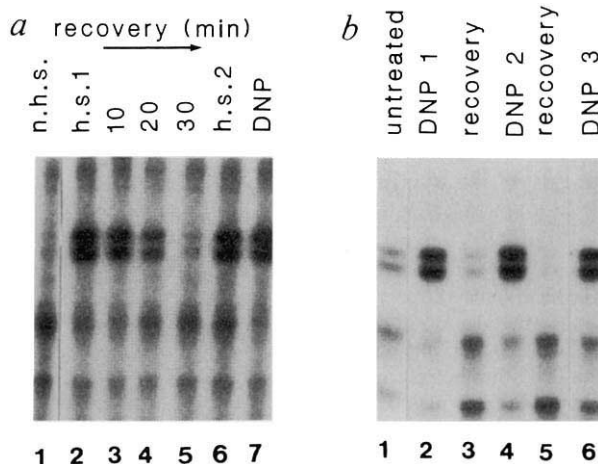


Fig. 2 Recovery and reinduction of HSE-binding activity. *ExoIII* protection assays were performed on extracts of cells given *a*, a heat shock or *b*, a DNP shock followed by sequential recovery and shock as indicated in the headings at the top.

Methods. For heat shock and recovery, SL2 cells ($5.4 \times 10^6 \text{ ml}^{-1}$) were heat shocked at 37.5°C for 10 min in a water bath. The cells were allowed to recover at 25°C with agitation (an absolute requirement). At the indicated times of recovery, 5-ml aliquots were transferred to a 50-ml culture tube and the cells were pelleted by centrifugation, resuspended in $\sim 1 \text{ ml}$ of the same medium, transferred to 1.5-ml microcentrifuge tubes, pelleted and frozen as in Fig. 1 legend. After 30 min of recovery, equal 5-ml aliquots were subjected to a second heat shock for 10 min or to treatment with 1 mM DNP (lane 7 in *a*) for 10 min; cells were pelleted and frozen. For DNP and shock recovery, 13 ml of cells ($1.5 \times 10^7 \text{ ml}^{-1}$) were treated with 1 mM DNP for 5 min at 25°C . The cells were pelleted at $200g$ for 3 min, washed once in fresh medium, pelleted, resuspended in fresh medium (original volume) and allowed to recover at room temperature with agitation for 15 min, before the next cycle of shock and recovery. At the end of each shock and each recovery, 1.5-ml aliquots of cells were pelleted and *ExoIII* assays were performed from whole cell extracts as in Fig. 1 legend.



observed by 30 s after transfer of cells to a 37.5°C bath. Considering the delay in temperature equilibration, the increase in specific binding is virtually instantaneous. The activity reaches a plateau (~ 20 -fold induction) by 5 min of shock and remains at that level after at least 1 h of continuous shock (data not shown). This increase in specific binding upon heat shock is also observed when KCl (up to 0.5 M) or NH_4SO_4 (0.36 M) are

employed instead of NaCl as the extracting salt in either whole-cell or nuclear extracts (data not shown). Figure 1a (right) shows that the maximal induced level of specific binding activity is directly related to the temperature elevation and unrelated to the duration of the shock: treatments at each temperature for 15 min (or more) do not result in higher binding activity. However, a low-temperature shock followed by a high-

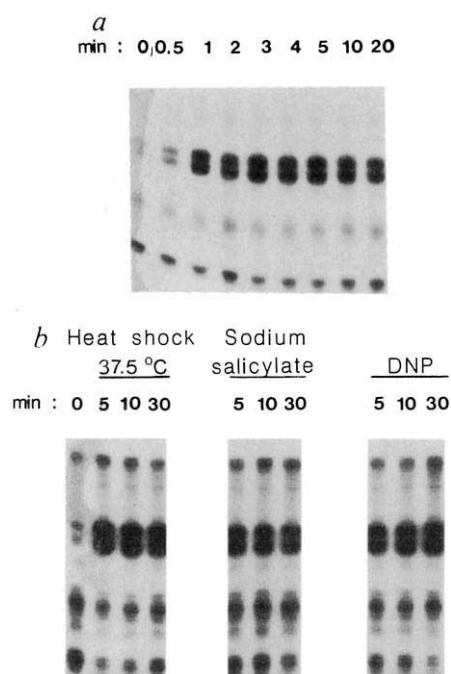


Fig. 3 Heat and chemical shock in absence of protein synthesis. *ExoIII* protection assays were performed on extracts of cells, *a*, heat shocked in the presence of cycloheximide and *b*, pretreated for 20 min with cycloheximide before heat or chemical shock in the continued presence of the drug as indicated in the headings at the top.

Methods. SL2 cells ($0.9 \times 10^7 \text{ ml}^{-1}$) were concentrated fourfold and were heat shocked at 37.5°C . Cycloheximide (Sigma, dissolved in phosphate buffered saline) was added to the culture (to $118 \mu\text{M}$) at time 0, and 0.5-ml aliquots were withdrawn at the indicated times; cell pellets, whole cell extracts, and *ExoIII* protection assays were performed as in Fig. 1 legend. This concentration of cycloheximide inhibits 95% of ^3H -leucine incorporation within the first 2 min of treatment and inhibits $>98\%$ incorporation thereafter, following standard procedures (S. Lindquist, personal communication) for protein synthesis studies (data not shown). For the pretreatment experiments, SL2 cells ($1.3 \times 10^7 \text{ ml}^{-1}$) were pretreated with $118 \mu\text{M}$ cycloheximide at 25°C for 20 min. In the continued presence of cycloheximide, 10 ml of cells were heat- or chemical-shocked, 1.5-ml aliquots were removed at the indicated times and pelleting of cells, preparation of whole cell extracts and *ExoIII* assays were performed as in Fig. 1 legend.

temperature shock does result in maximal DNA binding activity (data not shown). The differences in HSE-binding activity between normal and shocked cell extracts, shown previously¹¹ and further analysed here, contradict Parker and Topol's report that the HSE-binding protein (heat shock transcription factor) is present in nonshocked cells (the K_e line) at approximately the same levels as in heat shocked cells¹³. (It is possible that this discrepancy reflects a peculiarity of K_e cells or of the particular method of extract preparation, or that the cells were partially shocked during manipulation, and a saturating amount of factor was employed in the DNaseI footprinting assay.)

The induction of specific binding is also elicited by sodium salicylate and dinitrophenol (DNP) at concentrations similar to those which induce the formation of heat shock puffs^{1,2,16} (Fig. 1*b* and *c*). If the temperature or DNP stimulus is withdrawn, the binding activity decreases to basal levels within 30 min (Fig. 2*a*) (this is an upper estimate, because the cells were slowly re-equilibrated at room air temperature in this experiment). Further stimulation with heat or DNP reinduces specific binding to maximal levels. Figure 2*b* shows induction, recovery and reinduction of specific binding activity over three sequential cycles of DNP stimulus and withdrawal.

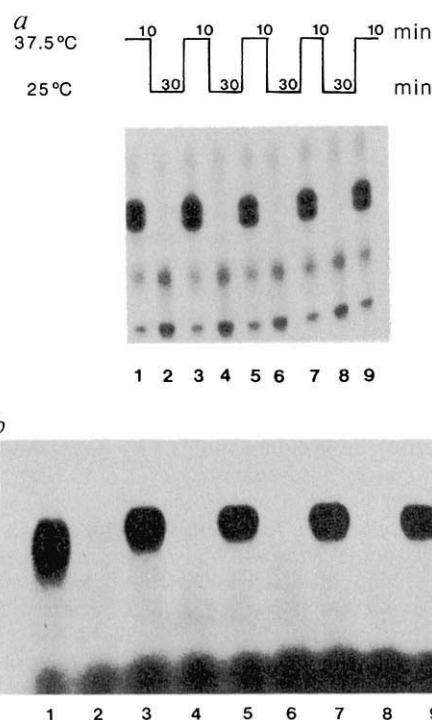


Fig. 4 Sequential induction, de-induction and reinduction of specific binding in absence of protein synthesis. *a*, *ExoIII* protection assays were performed on extracts of cells subjected to sequential heat shock (odd-numbered lanes) and recovery (even-numbered lanes) in the continuous presence of cycloheximide as indicated in the headings at the top. *b*, Gel retardation assays were performed on the same samples as in *a*; lane 10 is similar to lane 1 but includes 20 ng of unlabelled competitor HSE.

Methods. 16 ml of a fivefold concentration of SL2 cells (original density $5.6 \times 10^6 \text{ ml}^{-1}$) was treated with $118 \mu\text{M}$ cycloheximide, heat shocked immediately in a 37.5°C bath for 10 min and subsequently allowed to recover at 25°C for 30 min, with agitation in a shaker at 150 r.p.m. The process was repeated sequentially over five cycles, and 1-ml aliquots were removed at the end of each stimulus and each withdrawal. Cell pellets, whole cell extracts and *ExoIII* assays were performed as in Fig. 1 legend. Gel retardation assays were performed with 5 μl of each extract plus 4 μl of binding buffer (as in Fig. 1, except NaCl was 75 mM), followed by 6 μl of a mixture containing 1 μl of 10 mg ml^{-1} yeast tRNA, 1 μl of 1 mg ml^{-1} *Escherichia coli* DNA, 1 μl of 10 mg ml^{-1} pdN5, 1 μl of 50 mg ml^{-1} bovine serum albumin and 2 μl of 0.5 ng μl^{-1} ^{32}P -labelled heat shock element in the above binding buffer (final NaCl was about 150 mM). The heat shock element was prepared by annealing two oligonucleotides (non-coding strand: 5' GTCGACGGATCCGAGCGCCTCGAATGTTCTAGAAAAGG; the coding strand lacked the last 12 nucleotides from the 3' end and the duplex was labelled by filling in the single-stranded region with polymerase I (large fragment), [$\alpha\text{-}^{32}\text{P}$]dCTP, and unlabelled dTTP, dATP and dGTP, according to standard procedures. The binding reaction was allowed to occur for 10 min at 25°C , after which Ficol-400 was added to 2.5% (or alternatively glycerol to 5%) together with bromphenol blue and xylene cyanol dyes. The samples were electrophoresed on a 1% agarose gel (Seakem ME) in 45 mM Tris-borate EDTA buffer at room temperature for 1 h. The gel was dried on Whatman DE81 paper and autoradiographed.

Does the induction of specific binding require protein synthesis? Earlier experiments^{16,17} have indicated that protein synthesis is not required for the induction of chromosomal puffs. Figure 3 shows that the kinetics and extent of heat or chemical induction of specific binding activity is similarly unaffected by the protein synthesis inhibitor, cycloheximide, when over 98% of ^3H -leucine incorporation is suppressed. Similar results (not shown) are obtained with the inhibitor puromycin. In fact even

a 20 min pretreatment of SL2 cells with cycloheximide before the shock does not affect the induction of specific binding activity (the intensities of the protected fragments at maximal stimulus in Fig. 3 are directly comparable to those in Figs 1 and 2). These results suggest strongly that heat shock activator pre-exists in normal cells in a stable form which is unable to bind to the HSE with high affinity. This nonbinding form (form b) is converted upon shock to a high-affinity specific binding form (form a). There is some effect of cycloheximide on the induction of specific binding under mild temperature-shock and certain cell-growth conditions (data not shown), suggesting a secondary function for proteolysis in heat shock regulation, which has been advocated by several authors^{18,19}.

Is new protein synthesis necessary for reinduction of specific binding activity after a withdrawal period? The pattern of de-induction and reinduction of specific binding is also unaffected by the presence of cycloheximide (Fig. 4a). The pattern is retained even after five sequential cycles of heat shock and withdrawal over a period of 3 h. Similar results are observed over three cycles of DNP treatment (data not shown). We have reassayed the samples shown in Fig. 4a using the gel-retardation technique^{20,21}, and observe results fully consistent with the *ExoIII* protection assay (Fig. 4b). These results indicate that the (active) form, a, is not degraded during the recovery phase, but is processed back to the (inactive) form b, because maximal levels of form a can be reinduced by the second shock in the presence of cycloheximide. In fact, reinduction to maximal levels even after the fifth cycle makes it unlikely that form a, activated from a larger pool of form b, is degraded during each recovery phase. Evidently, heat shock activator protein is interconverted between active and inactive forms in dynamic response to cell shock and recovery. The conversion to HSE-binding could be due to release of a masking factor present in normal cells; however, an equal mixture of whole cell extracts from normal and heat shocked cells does not suppress HSE-binding activity (data not shown). It could be due to release of heat shock activator from an intracellular compartment, but the process of freeze-thaw, homogenization, and salt extraction of normal cells does not release significantly higher activity. Or it could be due to proteolytic activation from a larger precursor; however, the cycloheximide experiments argue against proteolysis as the primary mechanism for carrying out or mediating conversion to HSE-binding. Instead, we propose that a reversible post-translational modification of heat shock activator protein results in the HSE-binding activity. The nature of this conversion relates centrally to the heat shock induction mechanism and is currently under investigation. We note that activation by a post-translational modification has also been recently proposed for the nuclear protein NF- κ B (ref. 22).

We thank Susan Lindquist for leucine-free *Drosophila* culture medium and protocols for labelling SL2 cells, Mike Brownstein for preparing oligonucleotides, members of our laboratory and our colleagues in the Laboratory of Biochemistry for suggestions and assistance, and Beverly Miller for secretarial assistance.

Note added in proof: R. E. Kingston, T. J. Schuetz and Z. Larin (*Molecular and Cellular Biology* 7, 1530-1534 (1987)) have also found an inducible HSE-binding activity without protein synthesis in Hela cells.

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1. Ritossa, F. *Experientia* (Basel) **18**, 571-572 (1962).
2. Ritossa, F. M. *Expl Cell Res.* **35**, 601-607 (1964).
3. Ashburner, M. & Bonner, J. *J. Cell Biol.* **17**, 241-254 (1979).
4. Schlesinger, M. J., Ashburner, M. & Tissieres, A. (eds) *Heat Shock* (Cold Spring Harbor Laboratory, New York, 1982).
5. Lindquist, S. A. *Rev. Biochem.* **55**, 1151-1191 (1986).
6. Craig, E. *CRC crit. Rev. Biochem.* **18**, No. 3, 239-280 (1985).
7. Nover, L., Hellmund, D., Neumann, D., Scharf, K.-D. & Serfling, E. *Biologisches Zentralblatt* **103**, 357-435 (1984).
8. Schlesinger, M. J. *J. Cell Biol.* **103**, 321-335 (1986).
9. Pelham, H. R. B. *Cell* **30**, 517-582 (1982).
10. Wu, C. *Nature* **309**, 229-235 (1984).
11. Wu, C. *Nature* **311**, 81-84 (1984).
12. Wu, C. *Nature* **317**, 84-87 (1985).

13. Parker, C. & Topol, J. *Cell* **37**, 273-283 (1984).
14. Topol, J., Ruden, D. M. & Parker, C. *Cell* **42**, 527-537 (1985).
15. Munro, S. & Pelham, H. *Nature* **317**, 477-478 (1985).
16. Ashburner, M. *Chromosoma* (Berlin) **31**, 356-376 (1970).
17. Ritossa, F. M. & Pulitzer, J. F. *J. Cell Biol.* **19**, 60A (1963).
18. Ananthan, J., Goldberg, A. L. & Voellmy, R. *Science* **232**, 522-524 (1986).
19. Finley, D. & Varshavsky, A. *TIBS* **10**, 343-347 (1985).
20. Garner, M. M. & Revzin, A. *Nucleic Acids Res.* **9**, 3047-3060 (1981).
21. Fried, M. & Crothers, D. M. *Nucleic Acids Res.* **9**, 6505-6525 (1981).
22. Sen, R. & Baltimore, D. *Cell* **47**, 921-928 (1986).

A new class of synthetic antibacterials acting on lipopolysaccharide biosynthesis

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Although there is a need for antibacterial agents that act only on Gram-negative bacteria, there are at present few such compounds. The 2-deoxy analogue of β -KDO (3-deoxy- β -D-manno-2-octulopyranosonic acid) is a potent inhibitor of a key enzyme (CMP-KDO synthetase) in lipopolysaccharide biosynthesis of Gram-negative bacteria, but it fails to penetrate intact bacteria¹. Coupling an L-L-dipeptide to the 8-amino-2,8-dideoxy analogue of β -KDO enabled it to be recognized and actively accumulated by certain peptide permeases of the cytoplasmic membrane. The dipeptide was hydrolysed in the cell and the inhibitor released. Subsequent inhibition of CMP-KDO synthetase led to the accumulation of large amounts of lipid A precursor and bacterial death. These compounds represent a new class of synthetic antimicrobials with a novel mechanism of action and considerable potential as chemotherapeutic agents.

In any rational attempt to design antimicrobial agents effective against Gram-negative bacteria, the outer membrane appears as an attractive target. This structure is unique to Gram-negative bacteria and confers properties crucial to the survival of the organism in its natural habitat, as well as to its pathogenic potential and drug resistance^{2,3}. The outer membrane contains a unique molecule, lipopolysaccharide (LPS), consisting of three covalently linked regions³. Integrated into the outer membrane is the lipid A moiety, an acylated $\beta(1 \rightarrow 6)$ -linked glucosamine disaccharide². Extending outwards from the lipid A is the core, a short oligosaccharide. In most Gram-negative bacteria so far examined, the core oligosaccharide is linked to lipid A by an $\alpha(2 \rightarrow 6)$ -linked 3-deoxy-D-manno-2-octulosonate (KDO) dimer, to which may be linked a third KDO. In so called 'smooth' strains a long carbohydrate polymer, the O-side chain, extends from the core region^{2,3}.

Only one compound has been reported to be an inhibitor of LPS biosynthesis. The diazaborine 844674 (ref. 4) acts to block the incorporation of lipids into lipid A but is too toxic for use in chemotherapy. We identified the core sugar KDO as a promising target in the design of a novel antibacterial agent, because it is an essential component of LPS and mutants unable to produce KDO are non-viable⁵. In addition, as KDO is not present in mammalian cells², agents directed against KDO metabolism ought to be endowed with a high degree of selective toxicity.

The enzyme 3-deoxy-manno-octulosonate cytidyltransferase (CMP-KDO synthetase; EC 2.7.7.38) converts KDO to cytidine-5'-monophosphate-KDO (CMP-KDO). The substrate for a series of CMP-KDO-transferases able to incorporate KDO into the LPS. CMP-KDO synthetase is an essential gene product: temperature-sensitive mutations in CMP-KDO synthetase (*kdsB*) are lethal at the non-permissive temperature^{3,5}. A CMP-KDO assay⁶ was used to determine the inhibitory activity of a

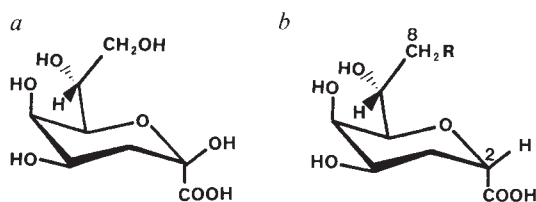


Fig. 1 *a*, Structure of 3-deoxy- β -D-manno-2-octulosonic acid (β -KDO) and *b*, inhibitors of CMP-KDO synthetase; R = $-\text{OH}$, 2,6-anhydro-3-deoxy-D-glycero-D-talo-octonic acid (dKDO); R = $-\text{NH}_2$, 8-amino-2,6-anhydro-3,8-dideoxy-D-glycero-D-talo-octonic acid. In the peptide derivatives R = L-alanyl-NH-, (Ala-NHdKDO), R = L-alanyl-L-alanyl-NH-, (Ala-Ala-NHdKDO) and R = L-prolyl-L-methionyl-NH-, (Pro-Met-NHdKDO).

series of KDO analogues synthesized in these laboratories. Although 2-deoxy- β -KDO (dKDO) (2,6-anhydro-3-deoxy-D-glycero-D-talo-octonic acid; Fig. 1) was found to be a potent inhibitor of CMP-KDO synthetase ($K_i = 3.9 \mu\text{M}$)¹, it was inactive against Gram-negative bacteria. The 8-amino-8-deoxy derivative of this inhibitor (NH₂-dKDO, 8-amino-2,6-anhydro-3,8-dideoxy-D-glycero-D-talo-octonic acid) possessed equivalent inhibitory activity against the isolated enzyme ($K_i = 4 \mu\text{M}$) and limited antibacterial activity (Table 1). The disappointing antibacterial effect was shown, by the use of radiolabelled inhibitors, to be due to the inability of these inhibitors to cross the cytoplasmic membrane in sufficient quantities to reach inhibitory concentrations at the target site (data not shown).

A dramatic increase in the uptake of other non-permeant enzyme inhibitors has been achieved by linking an amino acid or a short peptide to the molecule, thereby exploiting ubiquitous bacterial peptide permeases^{7,8} to transport the inhibitor through the cytoplasmic membrane^{9,10}. Transport forms of 2-deoxy- β -KDO were made by linking one or two amino-acid residues by a peptide bond to the 8-amino group of NH₂dKDO.

Such derivatives (Ala-NHdKDO, Ala-Ala-NHdKDO and Pro-Met-NHdKDO, failed to show inhibitory activity towards isolated CMP-KDO synthetase, presumably because the bulky 8-substituent prevented access to the active site of the enzyme. However, the dipeptide derivatives were potent bactericides against Gram-negative, but not Gram-positive bacteria (Table 1).

Suspensions of *Escherichia coli* strain ATCC 11303 were treated with 0.24 mM *N*-(L-alanyl-L-alanyl)-8-amino-2,6-anhydro-3,8-dideoxy-D-glycero-D-talo-[8-³H]octonic acid (Ala-Ala-NH[³H]dKDO), washed free of drug, extracted with boiling 5% toluene and the ³H-content of this cytoplasmic extract was determined. Ala-Ala-NH[³H]dKDO was taken up against the concentration gradient; after 5 min exposure the bacterium contained 28.3 mM drug rising to 52.6 mM at 30 min. Analysis of these extracts using HPLC (μ Bondapak-NH₂; with solvent acetonitrile/water, 65:35) failed to detect Ala-Ala-NH[³H]dKDO in the cytoplasm. A small amount (18%) was present after 30 min as Ala-NH[³H]dKDO but most of the activity (82%) was present as the liberated NH₂[³H]dKDO

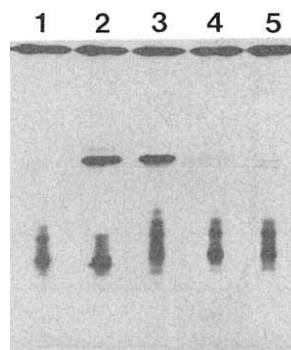


Fig. 2 Autoradiogram showing accumulation of lipid A precursor. *N*-acetyl-D-[1-¹⁴C]glucosamine (200 $\mu\text{Ci ml}^{-1}$, Amersham International) (8 μl) was added to 1-ml aliquots of *Salmonella typhimurium* AG701i50 (kdsA¹⁵). Samples were incubated at the permissive (30 °C) or non-permissive (42 °C) temperature or at 30 °C in the presence of 0.1 mM Ala-Ala-NHdKDO, Ala-NHdKDO or NH₂dKDO. After 30 min the incubation was stopped by adding 100 μl trichloroacetic acid (50% w/v), the tubes held on ice for 15 min and then centrifuged. The pellet was extracted with 50 μl butanol/pyridinium acetate (2:1), centrifuged and the supernatant applied to a silica gel TLC plate, which was developed using butanol/isobutyric acid/1 M NH₄OH (30:45:25). Contact autoradiography (24 h at 20 °C) revealed the presence at the permissive temperature (track 1) of several bands near the origin reflecting the heterogeneity of the lipid A present. At the non-permissive temperature (track 2), an additional band of radioactivity appears with an R_F equivalent to purified lipid A precursor (data not shown). When this band was removed from the plate it was found to be able to accept ³H-KDO from CMP-[³H]KDO in the presence of CMP-KDO lipid A transferase (data not shown). Treatment with Ala-Ala-NHdKDO (track 3) also led to accumulation of the lipid A precursor, whereas treatment with Ala-NHdKDO (track 4) or NH₂dKDO (track 5) did not. The precursor band could be detected at levels of Ala-Ala-NHdKDO or Pro-Met-NHdKDO that were as low as half the MIC, but was not induced by lethal levels of inhibitors of protein synthesis (chloramphenicol, tetracycline or streptomycin), peptidoglycan biosynthesis (bacitracin, penicillin G), or DNA synthesis (norfloxacin) (data not shown).

enzyme inhibitor. Ala-Ala-NH[³H]dKDO was also absent from extracts prepared after 5 min drug exposure, but in this case the proportion of free inhibitor was somewhat lower (71%). It appears that endogenous bacterial aminopeptidases¹¹ remove alanyl residues from Ala-Ala-NH[³H]dKDO during, or shortly after, drug uptake.

In enteric bacteria three systems for the uptake of peptides have been described¹²⁻¹⁴. Mutants defective in the dipeptide-, tripeptide-, or oligopeptide-permeases (*dpp*, *tpg* and *opp* mutants respectively) can be selected using resistance to bacilysin, alaphosphalin or triornithine respectively¹³. Permease-deficient mutants of *E. coli* (from J. W. Payne) were spread over the surface of peptide-free minimal media agar plates. Paper disks (6 mm diameter) containing 0.5 μmol Ala-Ala-NHdKDO were placed on the plates.

Table 1 Antibacterial activity of a KDO analogue and its transport forms

	Minimum inhibitory concentration (μM)						
	<i>E. coli</i> ATCC 11303	<i>Salmonella</i> <i>typhimurium</i> AG701i50	<i>Enterobacter</i> En 2	<i>Klebsiella</i> 150	<i>Staphylococcus</i> <i>aureus</i> ATCC 9144	<i>Bacillus</i> <i>subtilis</i> ATCC 6633	<i>Streptococcus</i> <i>faecalis</i> A1207
NH ₂ dKDO	i	2,000	i	i	i	i	i
Ala-NHdKDO	i	i	i	i	i	i	i
Ala-Ala-NHdKDO	8	6	120	250	i	i	i
Pro-Met-NHdKDO	2	1.5	120	250	i	i	i

Activity was determined by serial dilution of the drug in minimal (peptide-free) medium in microtitre plates, with a microbial inoculum of 10^3 bacteria per well and end points determined after 18 h incubation at the appropriate temperature (i, no activity at 2 mM).

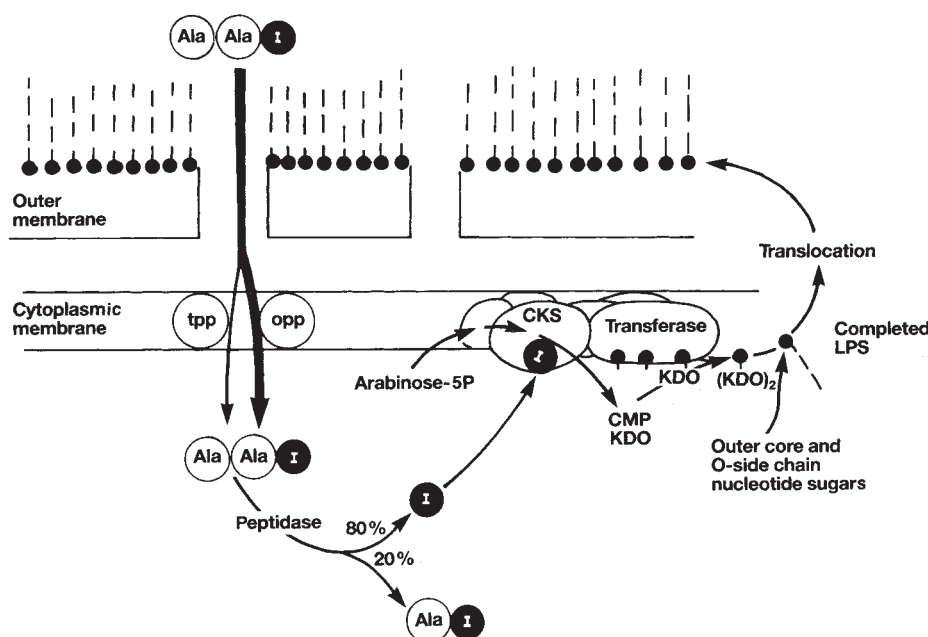


Fig. 3 Mechanism of action of dipeptide transport forms of a KDO analogue, CMP-KDO synthetase inhibitor in *E. coli*. Ala, Alanine; I, 8-amino-2,6-anhydro-3,8-dideoxy-D-glycero-D-talo-octonic acid; opp, tpp, oligo- and tripeptide permeases; CKS, CMP-KDO synthetase.

After overnight incubation at 37 °C on plates bearing a wild-type *E. coli* W (M26-26) possessing all three permeases, a zone of inhibition (diameter 38 mm) was seen around the disk. Zones of similar diameter were seen around disks placed on permease-deficient mutants of *E. coli* W such as PA 0113 (*dpp*⁻) and PA 0114 (*tpp*⁻), but on plates containing *E. coli* PA 0112 (*opp*⁻), PA 0123 (*dpp*⁻ *opp*⁻) and PA 0128 (*tpp*⁻ *opp*⁻) no inhibition zones were visible. Similarly, an *E. coli* K12 wild type was also sensitive (zone diameter 41 mm), but an *opp*⁻ derivative of it, PA 0183, was sensitive to some degree (zone diameter 13 mm). These data suggest that although the bulk of Ala-Ala-NHdKDO uptake in *E. coli* is by the oligopeptide permease, the compound may also be able to use, to a lesser extent, the *tpp* system of *E. coli* K12 strains.

In the biosynthesis of LPS the donation of KDO from CMP-KDO to an acylated and phosphorylated $\beta(1 \rightarrow 6)$ -linked D-glucosamine disaccharide known as lipid A precursor¹⁵⁻¹⁷ is an essential prerequisite for the secondary acylation of lipid A, incorporation of core and O-side chain sugars, and the translocation of LPS to the outer membrane^{2,3,15}. At the non-permissive temperature, although *kdsA* and *kdsB* mutants of *Salmonella typhimurium* are unable to produce CMP-KDO, synthesis of lipid A precursor continues unabated. Because the latter cannot be rapidly translocated to the cell exterior^{3,16}, it accumulates, inhibiting growth and ultimately causing the death of the bacterium^{3,15}. *S. typhimurium* AG 701150 (*kdsA*^{ts}) was grown at its permissive and non-permissive temperatures in the presence of N-acetyl-D-[1-¹⁴C]glucosamine, the LPS extracted and separated by TLC. Contact autoradiography (Fig. 2) revealed that at the non-permissive temperature large amounts of lipid A precursor accumulate at the expense of complete LPS. Treatment of the bacterium with Ala-Ala-NHdKDO or Pro-Met-NHdKDO also leads to the accumulation of lipid A precursor.

Lethal levels of these agents had no effect on protein, cell wall, RNA, or DNA synthesis, as measured by incorporation of radiolabelled leucine, diaminopimelic acid, uracil, or adenine into the relevant cellular macromolecules (data not shown). The diagram of LPS biosynthesis in *E. coli* shown in Fig. 3 indicates the mechanisms of drug uptake and hydrolysis and the consequences of subsequent inhibition of CMP-KDO synthetase.

We thank M. J. Osborn for mutants defective in KDO biosynthesis, J. W. Payne for help in determining the mechanism of

peptide uptake and C. Sahlberg for synthesizing the radioactive compounds.

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1. Claesson, A., Luthman, K., Gustafsson, K. & Bondesson, G. *Biochem. biophys. Res. Commun.* **143**, 1063-1068 (1987).
2. Hammond, S. M. in *The Bacterial Cell Surface* (eds Hammond, S. M., Lambert, P. A. & Rycroft, A. N.) 57-82 (Croom Helm, London, 1984).
3. Osborn, M. J. in *The Bacterial Outer Membrane* (ed. Inouye, M.) 15-34 (Wiley, New York, 1979).
4. Högenauer, G. & Woisetschlag, M. *Nature* **293**, 662-664 (1981).
5. Rick, D. & Osborn, M. J. *Proc. natn. Acad. Sci. U.S.A.* **69**, 3756-3760 (1972).
6. Ghalambor, M. A. & Heath, E. C. *J. biol. Chem.* **241**, 3216-3221 (1966).
7. Fickel, T. E. & Gilvarg, C. *Nature* **241**, 161-163 (1973).
8. Ames, B. N. et al. *Proc. natn. Acad. Sci. U.S.A.* **70**, 456-458 (1973).
9. Ringrose, P. S. in *The Scientific Basis of Antimicrobial Chemotherapy* (eds Greenwood, D. & O'Grady, F.) 219-281 (Cambridge University Press, 1985).
10. Berges, D. A. et al. *J. med. Chem.* **29**, 89-95 (1986).
11. Hemsdorf, C. L. & Simmons, C. in *Microorganisms and Nitrogen Sources* (ed. Payne, J. W.) 301-333 (Wiley, New York, 1980).
12. Gibson, M. M., Price, M. & Higgins, C. F. *J. Bact.* **160**, 122-130 (1984).
13. Higgins, C. F. & Gibson, M. M. *Meth. Enzym.* **125**, 365-377 (1986).
14. Payne, J. W. *Biochem. Soc. Trans.* **11**, 794-797 (1983).
15. Rick, P. D., Fung, W.-M., Ho, C. & Osborn, M. J. *J. biol. Chem.* **252**, 4904-4912 (1977).
16. Rick, P. D. & Young, D. A. *J. Bact.* **150**, 456-464 (1982).
17. Raetz, C. R. H., Purcell, S., Meyer, M. V., Quershi, N. & Takayama, K. *J. biol. Chem.* **260**, 16080-16088 (1985).

Errata

Electroreceptors in the platypus

J. E. Gregory, A. Iggo, A. K. McIntyre & U. Proske
Nature **326**, 386-387 (1987).

IN quoting the work of Scheich *et al.*, who measured the threshold voltage gradient for behavioural responses in the platypus, a value of 60 V cm⁻¹ was given. The value should have been 60 μ V cm⁻¹.

Abolition of the expression of inhibitory guanine nucleotide regulatory protein G_i in diabetes

D. Gawler, G. Milligan, A. M. Spiegel, C. G. Unson & M. D. Houslay
Nature **327**, 229-232 (1987).

AN error in the legend to Fig. 2 should be corrected as follows: Membranes are from mature animals in control (lane c), streptozotocin-diabetic (lane a) and streptozotocin-diabetic animals undergoing insulin therapy (lane b) states: DF, dye front.

Immunoassays for oncoproteins

J.P. Moore and G.I. Evan

The development of immunoassays for ras and myc proteins allows rapid and sensitive quantitation of oncoprotein expression in normal and transformed cells.

THE *ras* family (N-*ras*, Ki-*ras* and Ha-*ras*) and *myc* family (c-*myc*, N-*myc* and L-*myc*) of proto-oncogenes are components of the machinery that regulates cell growth. *Ras* proteins bind GTP and are involved in the transduction of the biochemical signals that initiate the growth of quiescent cells from growth factor receptors¹. Cells respond rapidly to these signals by increasing *c-myc* expression². Each of the *myc* proteins is located in the nucleus, but their function is uncertain, although several roles have been proposed for *c-myc*^{3,4}. Aberrant expression of *ras* and *myc* genes, either by the production of mutated proteins or the overproduction of normal proteins, has been implicated in the genesis of several human cancers⁵. The most sensitive method for assaying expression of these genes has been to analyse mRNA levels; this is laborious and provides no direct information about *ras* and *myc* protein levels. Alternatively, the oncoproteins themselves can be detected by immuno- (Western) blotting, immunoprecipitation, and by immunocytochemistry at the single cell level. These procedures are time-consuming, none is truly quantitative, and both Western blotting and immunoprecipitation are often insufficiently sensitive for detection of oncoproteins in non-transformed cells. However, recently developed immunoassays allow *ras* and *myc* proteins to be quantitated rapidly and simply^{6,7}. A radioimmunoassay for the P53 nuclear oncoprotein was described several years ago⁸.

The genesis of an assay

Horan Hand and colleagues have developed direct-binding liquid competition radioimmunoassays for *ras* oncogene and proto-oncogene products⁶. Essential constituents of the assays are several purified *ras* proteins expressed in bacteria (viral Ha-*ras* protein, human proto-Ha-*ras* protein and human T24 mutant Ha-*ras* protein), and monoclonal antibodies that recognize Ha- and Ki-*ras* proteins. Cell lysates, or extracts containing known amounts of purified *ras* protein, are incubated for 1 hour with a fixed amount of ¹²⁵I-labelled monoclonal antibody. The amount of antibody bound to *ras* protein at equilibrium is proportional to the amount of *ras* protein in the mixture. This mixture is then transferred to a microplate well containing adsorbed *ras* proteins, which bind any free antibody overnight. The ¹²⁵I-antibody on the plate is quantitated, and is inversely proportional to the amount of *ras* protein in the initial sample.

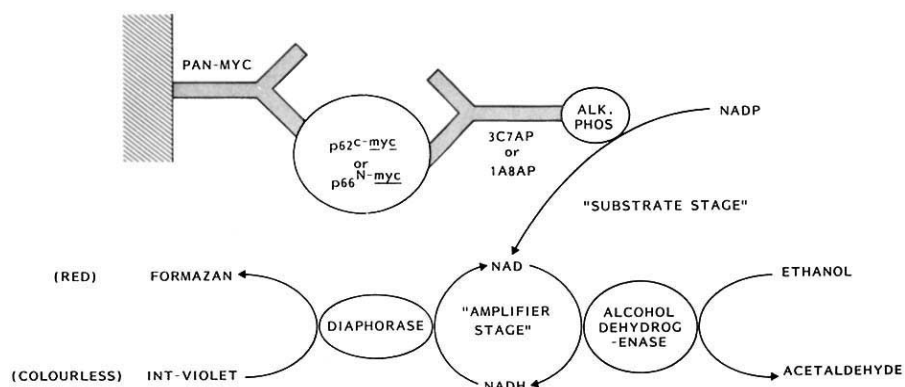


Fig. 1 Schematic representation of the twin-site ELISAs for the c-*myc* and N-*myc* oncoproteins, amplified with AMPAK (IQ (Bio) Ltd, Cambridge, UK). AP, alkaline phosphatase conjugate.

The *ras* assay detects purified *ras* protein in the range 0.1–1 ng. Concentrations of *ras* proteins in cell extracts are determined by establishing the amount of extract that prevents 50 per cent of the input ¹²⁵I-antibody from binding to the adsorbed *ras* proteins, and comparing this with a standard curve prepared using purified *ras* proteins. Molecules per cell values reported⁶ for total *ras* protein include 68,000 for NIH-3T3 fibroblasts, 680,000 for v-Ha-*ras* transfected fibroblasts, and 82,000 for T24 human bladder carcinoma cells containing mutated Ha-*ras* protein. The latter two *ras* transformed cells clearly contain very different amounts of *ras* protein: the transfected cells over-express *ras*, whereas the T24 cells have a “normal” level of abnormal protein.

Enzyme-linked immunosorbent assays (ELISA) for c-*myc* and N-*myc* proteins have also been developed recently⁷. These assays are somewhat simpler, quicker and more sensitive than the *ras* assays, and use a colorimetric detection system. Each uses two antibodies raised against different peptide sequences within the target protein. The binding of these anti-peptide antibodies to *myc* antigens is not conformation-dependent, and so very effective denaturants/solubilizing agents can be used to extract *myc* proteins from cells and tissues. The c-*myc* assay is calibrated by comparing signals obtained from cell lysates with those from known amounts of purified bacterially expressed c-*myc* protein (bp62^{c-myc})⁹.

The ELISAs have several essential components (Fig. 1). In the first step, pan-*myc* antibody¹⁰ is adsorbed to microtitre wells. This antibody has been raised against a peptide sequence substantially conserved in all known *myc* proteins and captures c-*myc*, N-*myc* and L-*myc* proteins from cell lysates, prepared by boiling

cells in SDS and dithiothreitol, alkylating with iodoacetamide and diluting in Nonidet P40. Captured human, murine or feline *myc* protein is then recognized at a distinct site by a specific anti-c-*myc* (myc1-3C7)¹¹ or anti-N-*myc* (Nmyc2-1A8) second monoclonal antibody conjugated to alkaline phosphatase. After washing, the amount of specifically bound alkaline phosphatase is proportional to the amount of input *myc* protein. Bound alkaline phosphatase is detected colorimetrically using an extremely sensitive enzymatic system¹² (AMPAK), which is summarized in Fig. 1. In the first stage, lasting 30 minutes, alkaline phosphatase dephosphorylates NADP to NAD; in the subsequent 5-minute amplification step, a pair of cycling redox reactions reduce a colourless precursor to a coloured formazan dye. The reaction is stopped with acid and the optical density determined at 492 nm using a standard plate reader.

Amplifying the signal

The sensitivity of the AMPAK system results from the amplification step. Each molecule of NAD is regenerated as the dye precursor is reduced. NAD is thus used catalytically rather than stoichiometrically, making AMPAK over 100 times more sensitive than conventional ELISA detection systems¹². The detection limit for bp62^{c-myc} is approximately 3pg, but maximum sensitivity is in the range 100 pg–1 ng⁷. The c-*myc* protein content of as few as 1,000–5,000 cells with an amplified c-*myc* gene, for example, HL60 promyelocytic leukaemia, can be measured, as can N-*myc* proteins in 10,000 neuroblastoma cells. Both N-*myc* and c-*myc* proteins are detectable in the same lysate of F9 or PSMB murine teratocarcinoma cells⁷.

The ELISA has been used to monitor changes in c-*myc* protein expression in

proliferating and differentiating cells of both human and murine origin, and to estimate the *c-myc* protein content of normal and transformed cells⁷. Molecules per cell values for *c-myc* protein could be obtained by ELISA within a few hours and were consistent with those from more conventional but much slower assays, such as Western blotting. Typical values ranged from < 1,000 in quiescent fibroblasts, to 7,000 in serum-stimulated fibroblasts, to 100,000–200,000 in various lymphoma and carcinoma cell lines with an amplified or translocated *c-myc* gene. In general, cell lines in which *c-myc* has been implicated in the genesis of the transformed phenotype contain 10–100-fold more *c-myc* protein than normal proliferating cells.

The principal disadvantage of the *myc* ELISAs, as in all immunoassays, is that the coloured end-product of the reaction provides less information than does a band on a gel or a filter. Nevertheless the ELISAs are specific: all signals are completely blocked by the peptides against which the antibodies were raised; and each of the antibodies recognizes only authentic *myc* proteins when used in immunoblotting or immunoprecipitation analyses^{7,10,11}.

The *myc* ELISAs should be of assistance to those investigating the functions of *myc* proteins in normal and transformed cell growth, and will simplify and speed the clinical screening of tumours for abnormal expression of *c-myc* and *N-myc* genes. It is perfectly feasible to screen several hundred cell lines or tumour specimens within a day by ELISA, and the extra sensitivity of the ELISAs should also enable serum and other body fluids to be assayed for *myc* proteins. The general principle of coupling specific anti-peptide antibodies to a sensitive detection system can, of course, be extended to create assays for a wide range of oncoproteins and other proteins of clinical interest. □

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1. Wakelam, M.J.O. *et al.* *Nature* **323**, 173–176 (1986).
2. Kelly, K., Cochran, B.H., Stiles, C.D. & Leder, P. *Cell* **35**, 603–610 (1983).
3. Studzinski, G.P., Brelvi, Z.S., Feldman, S.C. & Watt, R.A. *Science* **234**, 467–470 (1986).
4. Spector, D.L., Watt, R.A. & Sullivan, N.F. *Oncogene* **1**, 5–12 (1987).
5. Bishop, J.M. *Science* **235**, 305–311 (1987).
6. Horan Hand, P., Vilasi, V., Caruso, A. & Schlom, J. *Biochim. biophys. Acta* **908**, 131–142 (1987).
7. Moore, J.P., Hancock, D.C., Littlewood, T.D. & Evan, G.I. (in preparation).
8. Benchimol, S., Pim, D. & Crawford, L. *EMBO J.* **1**, 1055–1062 (1982).
9. Watt, R., Schatzman, A.R. & Rosenberg, M. *Molec. cell. Biol.* **5**, 448–456 (1985).
10. Evan, G.I., Ibsen, J., Hancock, D.C., Littlewood, T.D. & Waters, C.M. (in preparation).
11. Evan, G.I., Lewis, G.K., Ramsay, G. & Bishop, J.M. *Molec. cell. Biol.* **5**, 3610–3616 (1985).
12. Johansson, A., Ellis, D.H., Bates, D.L., Plumb, A.M. & Stanley, C.J. *J. immunol. Meth.* **87**, 7–11 (1986).

What counts in clinical chemistry

Next week, scientists will converge upon Ljubljana, Yugoslavia for the FEBS convention, and The Hague, the Netherlands for the XIII International Congress of Clinical Chemistry.

COLLECTING columnar cells from the endocervical canal is imperative in the diagnosis of *Chlamydia trachomatis* infections, but often cervical swabs just don't do the job. Now Syva has developed the Cytobrush, an **endocervical collection device** that it includes in its MicroTrak Direct Specimen Collection Kit (Reader Service No. 101). Syva says that during clinical trials, over 70 per cent more specimens collected with the Cytobrush were judged adequate than those collected with a swab. And when used with the MicroTrak test, the Cytobrush gives a statistically significant increase in the number of infections detected. Syva sells the Cytobrush in packs of 100 for £40 (UK), or as part of the £40 (UK) Specimen Collection Kit containing 20 tests. They will be on display at the XIII International Congress of Clinical Chemistry.

Beckman's new line of GP Series **table-top centrifuges** handles a wide variety of vessels, making them convenient accessories for clinical laboratories (Reader Service No. 102). The series is available



Soon Beckman's GP Series of centrifuges will be available as floor-standing models, too.

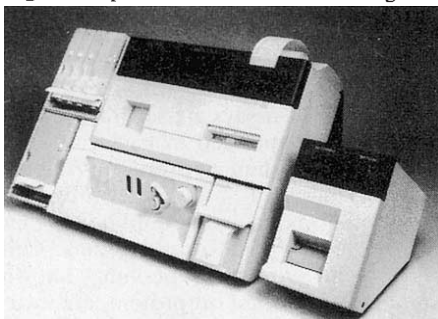
with or without refrigeration, and can spin up to three litres of samples at speeds of 3,750 r.p.m. and 3,200g with a \$1,100 (US) four-bucket horizontal rotor. With the same rotor, the centrifuge can also handle four 250 ml conical-tip tissue culture bottles or eight 96-well microplates. Blood bags are also likely candidates, as are large numbers of tubes in modular disk adapters: as many as 148 RIA, 76 serum separator or 56 15 ml tubes can be accommodated. Two 35° fixed angle rotors, rated at 6,400 r.p.m. and 5,642 g, can be used in addition to the horizontal rotor, and the series features automatic soft-start and a two-stage disk brake, to start off easy and end without resuspension. Without rotors, the GP series with refrigeration costs \$4,790 (US), and carries a \$2,925 (US) price tag without cooling.

Beckman will be exhibiting at both the FEBS and the clinical chemistry meetings.

Haematology helpers

A monoclonal antibody for use in blood-typing reagents is new from Celltech Ltd, exhibiting at the XIII International Congress of Clinical Chemistry (Reader Service No. 103). Celltech says BRIC-8 anti-C3d significantly improves the **detection of complement**, and produces a potent polyspecific anti-human globulin reagent when blended with polyclonal anti-human IgG, which gives fewer false positives than conventional reagents. Celltech recommends the use of BRIC-8 anti-C3d in direct Coombs and immediate spin tests, and sells 100 ml units for £1,300 (UK).

Samples actually pass through the sensing electrodes in Ciba Corning's new 200 Series **blood gas analysers**, eliminating the need for traditional membranes that require frequent replacement (Reader Service No. 104). The model 278 measures pH, pCO₂ and pO₂ plus nine additional parameters, while the model 280 also records a clinically significant haemoglobin value. For more sophisticated analyses, the model 288 comes with all of the above plus sodium and potassium detection, and a choice of ionized calcium or chloride detection. Results can be obtained with Ciba Corning's analysers in 45 to 60 seconds, and built-in memory storage allows recall of results and tracking of QC performance. Ciba Corning will



Ciba Corning's novel design for blood gas analyses eliminates downtime for membrane replacement.

have an exhibit at the XIII International Congress of Clinical Chemistry.

Becton Dickinson's LeucoPREP **blood cell separation tube** now comes in a larger 16 mm size that holds 10 ml of blood (Reader Service No. 105). The LeucoPREP tube has a sterile interior to permit

LeucoPREPTM Cell Separation Tube*

For the Separation of
Mononuclear Cells
from Whole Blood

STERILE INTERIOR

CAUTION:

Not for use in blood collection

5 ml Whole Blood (13 × 100 mm tube size)
LeucoPREP Tube Order No. 2750
10 ml Whole Blood (16 × 125 mm tube size)
LeucoPREP Tube Order No. 2751

FOR IN VITRO DIAGNOSTIC USE

SUMMARY AND EXPLANATION

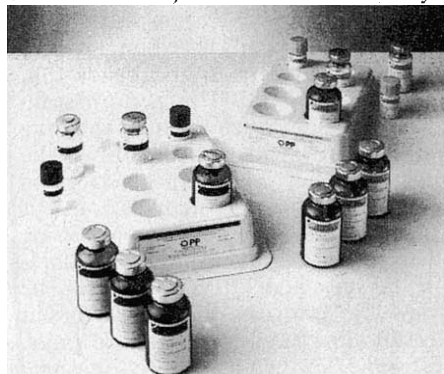
Isolation of mononuclear cells from whole blood is an important and necessary first step in many in vitro assays, including those in which immunofluorescence staining and functional assays are required. The currently accepted standard method for mononuclear

Now twice as large, Becton Dickinson's LeucoPREP tubes separate leukocytes from whole blood.

subsequent functional studies with separated cells, and was designed to eliminate the tedious Ficoll-Hypaque procedure for preparing peripheral blood mononuclear cell suspensions for immunofluorescence staining and analysis. Becton Dickinson says leukocytes can be separated out of anticoagulated whole blood by centrifuging for 10 to 15 minutes — four times faster than Ficoll-Hypaque with no difference in yield or purity. Exhibiting at the XIII International Congress of Clinical Chemistry, Becton Dickinson sells a box of 60 10 ml LeucoPREP tubes for \$120 (US).

Kits for the clinic

For the quick diagnosis of overdose patients, Porton Products will be launching new formulations of the Quantase assays for aspirin (salicylate) and acetaminophen (paracetamol) at the XIII International Congress of Clinical Chemistry (Reader Service No. 106). The colorimetric assays

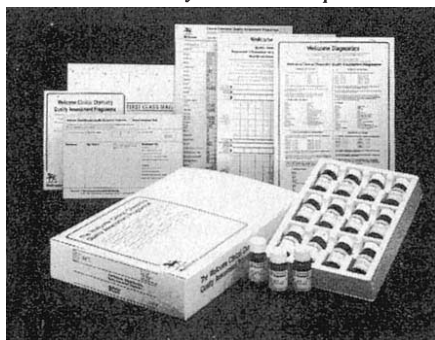


Patients who have overdosed on aspirin or acetaminophen can be diagnosed with Porton's kits.

are composed of only two reagents, the enzyme and the chromogenic agent, making them suitable for adaptation to a wide variety of discrete clinical chemistry analysers. Porton says the £30–40 (UK) tests take less than 15 minutes to

complete on an automatic analyser, and supplies method sheets to accompany the assays for all major discrete clinical instruments.

Wellcome Diagnostics will have plenty of information about its international **Quality Assessment Programmes** at the XIII International Congress of Clinical Chemistry (Reader Service No. 107). The programmes are designed to help clinical labs check up on their performance, and are conducted in six-monthly cycles. To participate, a lab sends in twelve dated specimens every two weeks, and receives back a computer analysis of quality that includes histograms, a Levey-Jennings chart showing progress through the cycle, and two summary charts for quick refer-



Wellcome's Quality Assessment Programme lets labs know about areas that need work.

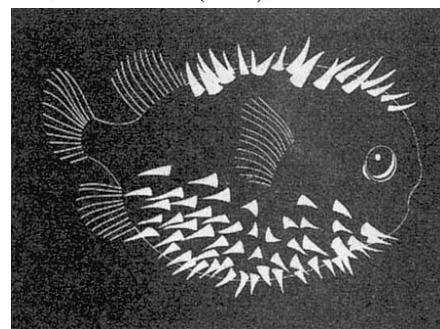
ence. At the end of the six-month period, Wellcome compiles a report providing an assessment of method precision and bias, and details of individual laboratory performance. The Wellcome Clinical Chemistry Programme measures up to 31 constituents, including 10 enzymes and common inorganic compounds. The Immunoassay Programme is split into two parts — one for immunoglobulins and steroid hormones, and one for thyroid status associated serum constituents, haematology constituents and tumour markers. Wellcome will be displaying its range of Wellcontrol Survey Validated Reference Sera, that are derived from the Quality Assessment Programmes, at the meeting.

Dako Corporation, a division of Dakopatts, has a new rabbit anti-human **prostatic acid phosphatase (PSAP)** antibody for research purposes that can stain tissue in 9 to 14 minutes (Reader Service No. 108). The anti-PSAP antibody Quick Staining Kit uses a labelled avidin-biotin detection system, that Dako says has been shown to be four to eight times more sensitive than the avidin-biotin complex system. The procedure involves incubating the tissue with: J1716 anti-PSAP for 1.5 minutes, biotinylated link antibody for 3 minutes, peroxidase labelled avidin for 1.5 minutes, and substrate solution for 3 minutes. The short incubation times with high-avidity primary and link antibodies eliminate the need for blocking of non-

specific protein binding and lengthy buffer baths. Dako's \$129 (US) Quick Staining Kit can be used with formalin-fixed paraffin-embedded tissue, and optional quenching and counterstaining steps can be added if desired. The 40-test kit detects PSAP in normal, benign, hypertrophic and neoplastic prostate ductal epithelial cells, labelling cytoplasm secretions and concretions. Dakopatts will have a product display at the clinical chemistry show.

Drug testing in the workplace has been an issue surrounded by controversy, and has become more prevalent in the last year. To **detect drugs of abuse** in urine, Roche Diagnostic Systems, a division of Hoffman-La Roche, has a line of diagnostic tests called Abuscreen EIA (Reader Service No. 109). The tests, based on an enzyme immunoassay, are available for the detection of cocaine, THC (the active ingredient in marijuana), barbiturates and morphine. The Abuscreen tests are performed in one test tube using the Roche COBAS RESA analysis system, and results are printed out in less than 20 minutes, according to Roche. Roche claims tests of several hundred clinical specimens at three different independent laboratories did not reveal any false negative or positive results through gas chromatography/mass spectrometry, because a wash step removes adulterants capable of skewing test results. Roche sells its Abuscreen EIA kits for \$200 (US) for 20 tests, and will have a display at the XIII International Congress of Clinical Chemistry.

For studies of disabling diseases involving muscles and nerves, Janssen offers a range of over 75 various **venoms and toxins**, including *Viperidae*, *Crotalidae*, *Elapidae*, scorpion, bee and toad venoms. Janssen also has the venom of the puffer fish, tetrodotoxin (TTX), that has been



The puffer fish is the source for Janssen's TTX venom — good for nerve and muscle research.

shown to be a specific sodium channel blocker in electrically excitable tissues (Reader Service No. 110). The TTX is available in 1 mg quantities with or without citrate buffer for £59 (UK), or coupled to tritiated lysine for £149 (UK). Janssen will have a stand at both meetings.

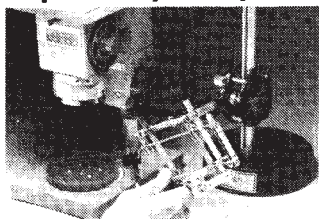
For analysing . . .

The centre of attraction in Vital Scientific's stand at the XIII International Congress of Clinical Chemistry will be its **VITALAB 200 random access patient profile analyser** (Reader Service No. 111). Vital Scientific says the VITALAB is the solution to performing a profile of tests on a few samples quickly, and can perform up to 12 tests at a time from a menu of 40 methods. The 12-position microcuvette block can either be filled by hand, or with the optional reagent loader that loads up to 20 different reagents under the control of the analyser. Process time per block depends upon the time required for the longest test, but Vital Scientific says 72 tests per hour can be achieved. Vital Scientific gears its range of analysers toward small- to medium-sized clinics, and has priced the VITALAB 200 accordingly, at Dfl 141,000 (the Netherlands).

Coulter will have its CPA Optimum Access **clinical analyser** and its DACOS 2 **Discrete sequential analyser** on display at

ADVERTISEMENTS

SINGER INSTRUMENTS Xenopus oocytes injection?



SINGER MKI MICROMANIPULATOR

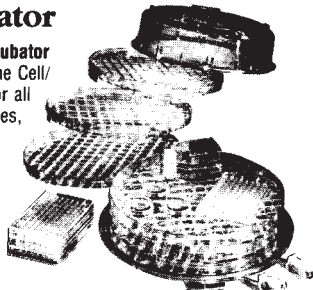
We also offer a Microsyringe Kit.

Write to:
Singer Instrument Co. Ltd.
Treborough Lodge
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Reader Service No.50

Versatile Tissue Culture Incubator

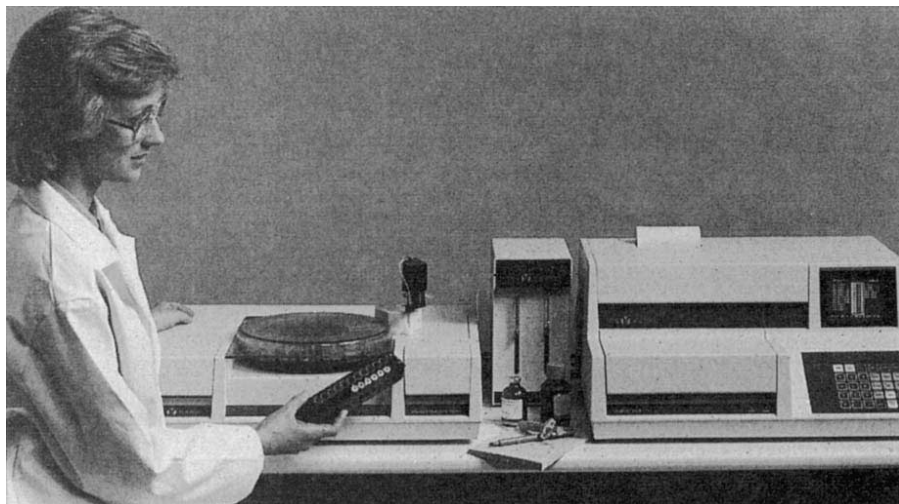
Modular Incubator Chamber (the Cell/Sphere™) for all tissue cultures, aerobic or anaerobic.

Low
Cost



Eliminates evaporation loss and contamination. Easy to use - flush with gas mixture, seal, place at appropriate temperature. Stackable, see-through. Quantity discounts. **billups-rothenberg, inc., pob 977, Del Mar, CA USA 92014.** Call 619-755-3309 or contact your Flow Laboratories representative.

Reader Service No.174



The patient profile analyser from Vital Scientific is priced for the small to medium-sized lab.

the XIII International Congress of Clinical Chemistry (Reader Service No. 112). The £27,000 (UK) CPA has automatic sample handling, microtest capability and level sensing for all reagents, says Coulter. The £104,480 (UK) DACOS 2 features primary sampling handling, bar code reading, and an on-board computer with 1.5 megabytes of expandable memory and 40 megabytes of storage capacity, sufficient for 32,000 patient records, that can be backed up on tape for security and archiving. The DACOS 2 is designed to perform up to 2,240 tasks without operator intervention, and produces 100 reports, with an average of six tests an hour.

Packard Instrument Company's COBRA is a benchtop Auto-Gamma **RIA counter** suited for labs with heavy workloads - it can count 1,000 tubes automatically, five or ten at a time, to accom-

pany are continuously monitored. Faulty detectors can be inactivated individually without disabling the entire counting block, and a quality control program warns users of impending failures and suggests corrective actions to avoid reruns or downtime. The COBRA's zigzag detector configuration virtually eliminates cross-talk for high-energy radionuclides, says Packard, and reduces background to levels of less than 30 c.p.m. for ¹²⁵I. Data can be merged with commercial spreadsheet or word processing software, or the COBRA's internal spreadsheet capability can be used. The COBRA is available for \$19,750 (US) with five detectors, or for \$26,250 (US) with ten. Packard will be at the Clinical Chemistry show.



Heavy-duty counting from Packard.

plish approximately 5,000 assays in one day (Reader Service No. 113). The brains behind its abilities is a built-in Packard PICO-XTE computer that can reduce and evaluate data according to any major curve calculation method selected by the user, and archive quality control data of 180 assay runs for each of 60 multiuser protocols. COBRA has an internal calibration package that calibrates all detectors during analysis, and instrument performance parameters such as detector resolution, background counts, detector counting efficiency and calibration accu-

Hunt-and-pecking on a keyboard is a thing of the past with the PU8700 **UV-visible spectrophotometer** from Philips (Reader Service No. 114). The system's operations can be controlled entirely through the use of a mouse, with on-screen horizontal softkeys, vertical pop-up menus and number pad windows. The £7,500-10,000 (UK) system consists of four main instruments based on a fixed 2 nm or variable 0.2 nm to 2 nm bandwidth. Philips says the PU8700 can perform scanning applications, such as blood and drug analyses, at 190-900 nm, and display the graphical result with a peak table in seconds. Colorimetric analyses, such as protein assays, can be achieved using either single- or multi-standard calibration curves, and the results can be reviewed in histogram or graphical format overlaid with preselected analytical limits. Accessories include an autocell, electrocell and cell programmer. Philips will have a stand at the XIII International Congress of Clinical Chemistry. □

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal and apply only within the country indicated.